```
=> d his 1

     (FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, WPIDS, SCISEARCH, AGRICOLA'
     ENTERED AT 07:53:32 ON 19 FEB 2004)
L19          66 DUP REM L18 (73 DUPLICATES REMOVED)

=> d que l19
L1           31 SEA KARSTEN T?/AU
L2         1377 SEA CURRIE M?/AU
L3         7959 SEA MOORE W?/AU
L4         9266 SEA (L1 OR L2 OR L3)
L5           89 SEA L4 AND (ENZYM?(5A) ACTIVIT?)
L6            8 SEA L5 AND ION(3A) EXCHANG?
L7       550550 SEA (MEASUR? OR DETECT? OR ASSAY? OR ANALY?)(5A)(ENZYM? OR
                ABZYM?)
L8         1024 SEA SEPARAT?(5A)(PRECURSOR# OR SUBSTRAT?)(5A) PRODUCT?
L9          115 SEA L8 AND (ION? OR ANION? OR CATION?)(5A) EXCHANGE?
L10         104 SEA L9 AND (ENZYM? OR ABZYM?)
L12         321 SEA L7 AND L8
L13          17 SEA L12 AND CHARGE#
L14          36 SEA L8 AND CHARGE#
L15          41 SEA L12 AND EXCHANGE
L16         139 SEA L6 OR L10 OR L13 OR L14 OR L15
L17           8 SEA L8 (5A) CHARGE?
L18         139 SEA L16 OR L17
L19          66 DUP REM L18 (73 DUPLICATES REMOVED)


=> d ibib abs l19 1-66

L19  ANSWER 1 OF 66  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER:     2003-363133 [34]   WPIDS
CROSS REFERENCE:      2003-093979 [08]; 2003-093980 [08]; 2003-201436 [19];
                      2003-210126 [20]; 2003-210323 [20]; 2003-289681 [28]
DOC. NO. NON-CPI:     N2003-290022
DOC. NO. CPI:         C2003-095883
TITLE:                A method of chemical analysis useful for assaying
                      compounds simultaneously against many enzyme
                      -substrate pairs, comprises combining one test compound
                      with a solution comprising m enzymes and n substrates
                      complementary to the enzyme.
DERWENT CLASS:        B04 D16 S03
INVENTOR(S):          CHIEM, N H; GILBERT, J R; GILBERT, J
PATENT ASSIGNEE(S):   (COVE-N) COVENTOR INC
COUNTRY COUNT:        100
PATENT INFORMATION:

     PATENT NO   KIND DATE     WEEK      LA   PG
     -------------------------------------------
     WO 2003025115 A1 20030327 (200334)* EN   13
        RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
            MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
     US 2003077570 A1 20030424 (200334)

APPLICATION DETAILS:
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PATENT NO	KIND	APPLICATION	DATE
WO 2003025115 A1		WO 2002-US21438	20020708
US 2003077570 A1	Provisional	US 2001-323962P	20010920
		US 2001-27922	20011221

PRIORITY APPLN. INFO: US 2001-29108 20011221; US 2001-323962P 20010920; US 2001-27484 20011221; US 2001-27516 20011221; US 2001-27922 20011221; US 2001-28852 20011221

AN 2003-363133 [34] WPIDS

CR 2003-093979 [08]; 2003-093980 [08]; 2003-201436 [19]; 2003-210126 [20]; 2003-210323 [20]; 2003-289681 [28]

AB WO2003025115 A UPAB: 20030529

NOVELTY - A method of chemical analysis comprising combining one test compound with a solution comprising m enzymes and n substrates complementary to the enzyme, where m is 1 or greater, n is 1 one or greater, and m + n at least 3.

DETAILED DESCRIPTION - A method of chemical analysis comprising:

(a) combining one test compound with a solution comprising m enzymes and n substrates complementary to the enzyme, where m is 1 or greater, n is 1 one or greater, and m + n at least 3;

(b) incubating the test compound within the solution;

(c) separating the chemical species in the combined solution by chromatography; and

(d) measuring the relative amounts of **substrates** and **separately** identifiable **products** produced by a chemical reaction catalyzed by the enzymes, where chromatography is carried out within a microfluidic device.

USE - The method is useful for **assaying** compounds simultaneously against many **enzyme**-substrate pairs to identify compounds, which are potent inhibitors or stimulators of an enzyme.

ADVANTAGE - The advantage of the new method over separate assays in multiple wells are that experimental results are not degraded by variation of test compound concentration from well to well, and that one capillary electrophoresis channel **separation** can measure all the **substrate/product** pair ratios in one relatively quick experiment, thus showing improved efficiency for high throughput screening. Furthermore, the method allows the screening for the presence of an enzyme in a biological sample containing multiple enzymes without having to purify the enzyme first.

Dwg.0/0

L19 ANSWER 2 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER:	2003-221527 [21] WPIDS
CROSS REFERENCE:	1999-059777 [05]; 2003-310955 [30]; 2003-479443 [45]; 2004-051527 [05]
DOC. NO. CPI:	C2003-056298
TITLE:	Bacterial expression system for producing recombinant human uteroglobin for treating inflammatory and fibrotic conditions, comprises a synthetic gene which codes for human uteroglobin.
DERWENT CLASS:	B04 D16
INVENTOR(S):	PILON, A L; WELCH, R W; WELCH, R E
PATENT ASSIGNEE(S):	(PILO-I) PILON A L; (WELC-I) WELCH R W; (CLAR-N) CLARAGEN INC
COUNTRY COUNT:	100

Search completed by David Schreiber 308-4292

Gitomer 09/888,008

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003003979	A2	20030116	(200321)*	EN	64

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW

US 2003109429 A1 20030612 (200340)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003003979	A2	WO 2002-US20836	20020702
US 2003109429	A1 CIP of	US 1997-864357	19970528
		US 2001-898616	20010702

FILING DETAILS:

PATENT NO	KIND	PATENT NO
US 2003109429	A1 CIP of	US 6255281

PRIORITY APPLN. INFO: US 2001-898616 20010702; US 1997-864357 19970528

AN 2003-221527 [21] WPIDS

CR 1999-059777 [05]; 2003-310955 [30]; 2003-479443 [45]; 2004-051527 [05]

AB WO2003003979 A UPAB: 20040120

NOVELTY - A bacterial expression system for producing recombinant human uteroglobin (rhUG) comprising a synthetic gene which codes for human UG and which comprises any of the 4 nucleotide sequences given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) producing rhUG research seed bank, rhUG master cell bank, or rhUG production cell bank;

(2) expressing rhUG;

(3) purifying rhUG;

(4) producing a pharmaceutical grade rhUG drug substance;

(5) assay methods for determining the potency of rhUG in a sample;

(6) measuring in vitro the anti-inflammatory effect arising from inhibition or blocking of secretory phospholipase A2 **enzymes** by rhUG;

(7) assaying for the inhibition of secretory phospholipase A2 activity by rhUG;

(8) measuring in vitro the binding of rhUG to fibronectin;

(9) determining the purity of rhUG; and

(10) a pharmaceutical composition comprising a purified rhUG and optionally, a carrier or diluent.

ACTIVITY - Antiinflammatory; Antiasthmatic; Nephrotropic; Antirheumatic; Antiarthritic.

No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The bacterial expression system is useful in producing purified recombinant human uteroglobin for the treatment of inflammatory and

fibrotic conditions, such as neonatal respiratory distress syndrome and bronchopulmonary dysplasia. The rhUG may also be used for treating other conditions associated with elevated phospholipase A2 levels such as pancreatitis, acute renal failure, rheumatoid arthritis or asthma. The method is also useful for evaluating the relative strength of in vivo biological activity of rhUG.
Dwg.0/33

L19 ANSWER 3 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2003-247933 [24] WPIDS
DOC. NO. NON-CPI: N2003-197075
DOC. NO. CPI: C2003-063797
TITLE: Formation of battery separator, used in alkali secondary batteries, comprises graft copolymerizing 2-acrylamido-2-methyl-propane sulfonic acid and monomer with carboxy groups to substrate comprising polyolefin fibers.
DERWENT CLASS: A85 L03 X16
INVENTOR(S): KANENORI, J; SHIMANO, Y
PATENT ASSIGNEE(S): (KOMS) KOMATSU SEIREN KK
COUNTRY COUNT: 99
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003003486	A1	20030109	(200324)*	JA	53
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW					
JP 2003151523	A	20030523	(200343)		20

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003003486	A1	WO 2002-JP2657	20020320
JP 2003151523	A	JP 2002-24136	20020131

PRIORITY APPLN. INFO: JP 2002-24136 20020131; JP 2001-168451 20010604; JP 2001-266351 20010903
AN 2003-247933 [24] WPIDS
AB WO2003003486 A UPAB: 20030410
NOVELTY - Battery separator is formed by graft copolymerizing 2-acrylamido-2-methyl-propane sulfonic acid and a monomer with carboxy groups to a substrate comprising polyolefin fibers.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for the **production** of a battery **separator** by immersing a polyolefin fiber **substrate** in a monomer containing 2-acrylamido-2-methyl-propane sulfonic acid and a monomer with carboxylic acid groups, and applying high energy irradiation to the substrate in a state where the substrate treated with the monomer solution is interposed with the material which retains the substrate and the polyester film.
USE - The separator is used in alkali secondary batteries using nickel hydroxide as a positive electrode active material (claimed).
ADVANTAGE - The ammonia capturing capacity of the separator is

increased and the self-discharge of a battery can be suppressed. The capacity retention is high and the cycle of repeated **charge** and discharge is increased.

DESCRIPTION OF DRAWING(S) - Figure 1 shows the weight fraction of graft matter of the separator and the relationship of IEC(cal), IEC(obs), IEC(cal)-IEC(obs) and ammonia theoretical capture amount ATC. (Drawing contains non-English language text).

Dwg.1/1

L19 ANSWER 4 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: 2004-051527 [05] WPIDS
CROSS REFERENCE: 1999-059777 [05]; 2003-221527 [21]; 2003-310955 [30]; 2003-479443 [45]
DOC. NO. NON-CPI: N2004-041618
DOC. NO. CPI: C2004-020900
TITLE: Bacterial expression system for production of recombinant human uteroglobin comprising synthetic gene or human cDNA sequence which codes for human uteroglobin.
DERWENT CLASS: B04 D16 K08 S03
INVENTOR(S): PILON, A L; WELCH, R W
PATENT ASSIGNEE(S): (PILO-I) PILON A L; (WELC-I) WELCH R W
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2003207795	A1	20031106	(200405)*		64

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2003207795	A1 CIP of	US 1997-864357	19970528
	CIP of	US 2001-898616	20010702
		US 2002-187498	20020702

FILING DETAILS:

PATENT NO	KIND	PATENT NO
US 2003207795	A1 CIP of	US 6255281

PRIORITY APPLN. INFO: US 2002-187498 20020702; US 1997-864357 19970528; US 2001-898616 20010702

AN 2004-051527 [05] WPIDS
CR 1999-059777 [05]; 2003-221527 [21]; 2003-310955 [30]; 2003-479443 [45]
AB US2003207795 A UPAB: 20040120

NOVELTY - Bacterial expression system for the production of recombinant human uteroglobin (rhUG), comprising a synthetic gene or human cDNA sequence which codes for human UG, is new.

DETAILED DESCRIPTION - Bacterial expression system (I) for the production of recombinant human uteroglobin (rhUG), comprising a synthetic gene having a sequence of 5'-GATCCATGGAAATCTGCCCGTCTTTCCAGCGTGTTATCGAAACCC TGCTGATGGACACCCCGTCC-3', 5'-AGCTACGAAGCAGCTATGGAACTGTTCTCTCCGGACCAGGACATGC GTGAAGCAGGTGCT-3', 5'-CAGCTGAAGAAACTGGTTGACACCCTGCCGCAGAAACCGCGTGAATCCATCA TAAACTG-3', and 5'-ATGGAGAAGATCGCTCAGTCTAGCCTGTGCAACTAAG-3', or human cDNA sequence which further comprises Met-Ala-Ala at the N-terminus of the sequence, where the above mentioned sequences codes for human UG.

INDEPENDENT CLAIMS are included for the following:

(1) producing (M1) a rhUG master cell bank,comprising:
(a) inoculating a suitable incubating broth with an aliquot portion of a rhUG research seed bank to form a bacterial culture;
(b) incubating the bacterial culture;
(c) adding a cryopreservative to the bacterial culture to form a cryopreserved solution;
(d) transferring a portion of the cryopreserved solution to a cryovial; and
(e) storing the cryovial at a temperature below -60 deg. C;
(2) expressing (M2) rhUG, comprising:
(a) providing a production seed cell bank culture comprising an expression vector capable of expressing rhUG;
(b) inoculating a broth medium with the production seed cell bank culture to form an inoculum;
(c) incubating the bacterial culture formed in step (b);
(d) inoculating a large scale fermenter with the inoculum formed from the step (c) to form a fermentation culture;
(e) incubating the fermentation culture within the large scale fermenter;
(f) adding an induction agent to the fermentation culture to induce the expression of rhUG; and
(g) harvesting the above fermentation culture,
(3) expressing (M2) rhUG, comprising:
(a) inoculating a large scale fermenter with an inoculum comprising an expression vector capable of expressing rhUG to form a fermentation culture;
(b) incubating the fermentation culture within the large scale fermenter;
(c) adding an induction agent to the fermentation culture to induce the expression of rhUG; and
(d) harvesting the fermentation culture;
(4) purifying (M3) rhUG, comprising:
(a) providing a bacterial cell paste comprising bacterial cells capable of overexpressing rhUG;
(b) lysing the bacterial cell paste to form a supernatant;
(c) filtering the supernatant through a first nominal molecular weight cut off (NMWCO) membrane to form a first permeate;
(d) concentrating the first permeate by the use of a second NMWCO membrane;
(e) loading the concentrated permeate onto an **anion exchange** column to form a first eluate;
(f) concentrating the first eluate by the use of a third NMWCO membrane to form a second concentrate;
(g) loading the second concentrate onto a Hydroxyapatite (HA) column to form a second eluate;
(h) separating host-derived proteins from the rhUG in the second eluate to provide purified rhUG; and
(i) recovering the above purified rhUG, or
(5) purifying (M3) rhUG, comprising:
(a) providing bacterial cells capable of overexpressing rhUG;
(b) lysing the bacterial cells to form a supernatant liquid;
(c) filtering the liquid through a molecular weight cut off (NMWCO) membrane;
(d) loading the liquid onto an exchange column;
(e) separating host-derived proteins from the rhUG to provide purified rhUG; and
(f) recovering the purified rhUG;
(6) determining (M4) the potency of rhUG in a sample, comprising:
(a) contacting a sample containing rhUG with phospholipase A2;

(b) introducing fluorescently labeled substrate to the sample, separating the product or non-cleaved substrate from the sample; and

(c) determining level of cleavage or amount of cleaved substrate by fluorescence;

(7) measuring (M5) in vitro anti-inflammatory effect arising from inhibition or blocking of secretory phopsholipase A2 enzymes by rhUG, comprising:

(a) contacting the sample containing rhUG with phospholipase A2;

(b) introducing labeled substrate to the sample, separating product from sample; and

(c) determining level of cleavage by scintillation counting;

(8) measuring (M6) in vitro binding of rhUG to fibronectin, comprising:

(a) contacting a recombinant fragment of human fibronectin with a recombinant human CC10-horse radish peroxidase (HRP) conjugate; and

(b) visualizing the assay to determine binding of rhUG to the fibronectin fragment;

(9) determining (M7) purity of rhUG, comprising taking samples of intermediates at each step of (M3) and analyzing the process intermediates; and

(10) a pharmaceutical composition (II) comprising a purified rhUG of (M3), or rhUG and a carrier or diluent.

ACTIVITY - Antiinflammatory.

MECHANISM OF ACTION - Inhibitor of secretory phospholipase A2; Binds to fibronectin (claimed).

The binding of rhUG (cGMP lots of rhUG such as rhCC10) to fibronectin was performed as follows. rhCC10 prevented inappropriate deposition and subsequent formation of a pro-fibrotic extracellular matrix. Microtiter plates were coated with the fibronectin fragment overnight and binding of rhCC10 was detected by competition with a rhCC10-HRP conjugate. RhCC10-HRP conjugate was added to the plates and incubated for 1 hour at room temperature. The conjugate was added with or without standard or sample. Phosphate buffered saline (PBS) was used as a negative control. The plate was aspirated and washed four times. The assay was visualized by the o-phenyldiamine dihydrochloride (OPD) HRP assay from Pierce. The plate was read at 490 nm using a Biotek EL-80 microplate reader and the data was analyzed using Biotek KC4 software. The results of this assay showed that cGMP lots of rhUG was positive for binding of rhUG to the fibronectin fragment.

USE - (I) is useful for producing a rhUG research seed bank or a pharmaceutical grade rhUG drug substance. (II)-(b) having rhUG is safe to administer to a patient in respiratory distress. (All claimed.) The rhUG is useful for treating inflammation and fibrotic diseases.

DESCRIPTION OF DRAWING(S) - The drawing shows the construction of synthetic bacterial gene for recombinant human uteroglobin.

Dwg.1/33

L19 ANSWER 5 OF 66 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2003443561 MEDLINE
DOCUMENT NUMBER: 22866160 PubMed ID: 14504677
TITLE: Biochemical analysis with microfluidic systems.
AUTHOR: Bilitewski Ursula; Genrich Meike; Kadow Sabine; Mersal Gaber
CORPORATE SOURCE: Dept. Natural Products Biology, Gesellschaft fur Biotechnologische Forschung mbH, Mascheroder Weg 1, 38124, Braunschweig, Germany.. ubi@gbf.de
SOURCE: Anal Bioanal Chem, (2003 Oct) 377 (3) 556-69. Ref: 97
Journal code: 101134327. ISSN: 1618-2642.
PUB. COUNTRY: Germany: Germany, Federal Republic of

Search completed by David Schreiber 308-4292

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 20030924
Last Updated on STN: 20031113
Entered Medline: 20031112

AB Microfluidic systems are capillary networks of varying complexity fabricated originally in silicon, but nowadays in glass and polymeric substrates. Flow of liquid is mainly controlled by use of electroosmotic effects, i.e. application of electric fields, in addition to pressurized flow, i.e. application of pressure or vacuum. Because electroosmotic flow rates depend on the **charge** densities on the walls of capillaries, they are influenced by substrate material, fabrication processes, surface pretreatment procedures, and buffer additives. Microfluidic systems combine the properties of capillary electrophoretic systems and flow-through analytical systems, and thus biochemical analytical assays have been developed utilizing and integrating both aspects. Proteins, peptides, and nucleic acids can be separated because of their different electrophoretic mobility; detection is achieved with fluorescence detectors. For protein analysis, in particular, interfaces between microfluidic chips and mass spectrometers were developed. Further levels of integration of required sample-treatment steps were achieved by integration of protein digestion by immobilized trypsin and amplification of nucleic acids by the polymerase chain reaction. Kinetic constants of enzyme reactions were determined by adjusting different degrees of dilution of enzyme substrates or inhibitors within a single chip utilizing mainly the properties of controlled dosing and mixing liquids within a chip. For analysis of kinase reactions, however, a combination of a reaction step (enzyme with **substrate** and inhibitor) and a **separation** step (enzyme **substrate** and reaction **product**) was required. Microfluidic chips also enable separation of analytes from sample matrix constituents, which can interfere with quantitative determination, if they have different electrophoretic mobilities. In addition to **analysis** of nucleic acids and **enzymes**, immunoassays are the third group of analytical assays performed in microfluidic chips. They utilize either affinity capillary electrophoresis as a homogeneous assay format, or immobilized antigens or antibodies in heterogeneous assays with serial supply of reagents and washing solutions.

L19 ANSWER 6 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:358546 BIOSIS
DOCUMENT NUMBER: PREV200300358546
TITLE: Assay development and high throughput screening with an inositol 5'-phosphatase on a microfluidic LabChip(R) platform.
AUTHOR(S): Zhou, Aileen [Reprint Author]; Panfili, Peter; Rowe, Todd; Kurzeja, Rob; Menjares, Anthony; McCarter, John
CORPORATE SOURCE: Applications, Caliper Technologies Corp, 605 Fairchild Dr., Mountain View, CA, 94043, USA
aileen.zhou@calipertech.com; peter.panfili@calipertech.com; rowe@amgen.com; kurzeja@amgen.com; menjares@amgen.com; mccarter@amgen.com
SOURCE: FASEB Journal, (March 2003) Vol. 17, No. 4-5, pp. Abstract No. 845.17. http://www.fasebj.org/. e-file.
Meeting Info.: FASEB Meeting on Experimental Biology:

Search completed by David Schreiber 308-4292

Translating the Genome. San Diego, CA, USA. April 11-15, 2003. FASEB.
ISSN: 0892-6638 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Aug 2003
Last Updated on STN: 6 Aug 2003

AB Inositol 5'-phosphatases are a family of enzymes that play a critical role in mediating signaling via lipid and soluble inositol phosphates by specifically catalyzing the hydrolysis of the 5'-phosphate of the inositol moiety. Previously reported **assays** for such **enzymes** typically involve separation of a radiolabeled version of a phosphatidylinositol lipid substrate from the dephosphorylated product. We have developed an alternative assay amenable to high throughput screening in an off-chip mobility shift assay format using Caliper LabChip(R) technology. BODIPY(R) FL-phosphatidylinositol 3,4,5-triphosphate (BODIPY-PIP3) was used as a substrate for this 5'-phosphatase. The triphosphate substrate bears a -7 **charge**, while the diphosphate product bears a -5 **charge**, facilitating separation of the two based upon their different electrophoretic mobilities. Optimal conditions for **separation** of **substrate** and **product** were established, and the kinetics of enzymatic conversion were evaluated. Incubation of enzyme (0.5apprx1 nM) with substrate (1 mM) for 2 hours resulted in apprx 50% substrate conversion. The Km of the PIP3 substrate was determined to be 135mM, and kcat/Km was found to be apprx 2x10e5 M-1s-1. Dose-dependent inhibition was demonstrated with the classical phosphatase inhibitors bpV(phen) and mpV(pic). A 40,000 compound library was screened and hits were confirmed.

L19 ANSWER 7 OF 66 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2002:450256 HCAPLUS
DOCUMENT NUMBER: 137:2733
TITLE: **Ion-exchange** resin / **enzyme activity assay**
INVENTOR(S): **Karsten, Thomas P.; Currie, Mark G. ; Moore, William M.**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 7 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002072082	A1	20020613	US 2001-888008	20010622

PRIORITY APPLN. INFO.: US 2000-213354P P 20000622

AB The present invention relates to a rapid high-throughput **ion-exchange** resin **assay** for determining **enzyme activity**. This novel assay uses a radiometric technique which **separates** the radioactive **substrate** from the **product** (or the radioactive product from the substrate) by exploiting the differences in the net **charges** of the mols. using **ion-exchange** resin. This assay is useful, for example, for studies of **enzyme** kinetics, the identification of functional sites in the **enzyme**, and in the automated screening of compound libraries for pharmaceutical drug development.

L19 ANSWER 8 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-674850 [72] WPIDS
CROSS REFERENCE: 1997-393613 [36]; 1998-322748 [28]; 1998-557036 [47]; 2002-083110 [11]; 2002-750464 [81]; 2003-596420 [56]; 2003-697784 [66]
DOC. NO. CPI: C2002-190055
TITLE: Composition useful for e.g. separation of nucleic acids comprises a positively or neutrally **charged** phosphoramidite.
DERWENT CLASS: B04 B05 D16
INVENTOR(S): ALLAWI, H T; LYAMICHEV, V; NERI, B P; SKRZPCZYNSKI, Z; TAKOVA, T; WAYLAND, S R
PATENT ASSIGNEE(S): (THIR-N) THIRD WAVE TECHNOLOGIES INC
COUNTRY COUNT: 101
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002063030	A2	20020815	(200272)*	EN	197
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW					
US 2002128465	A1	20020912	(200272)		
EP 1385996	A2	20040204	(200410)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2002063030	A2		WO 2002-US3423	20020206
US 2002128465	A1	CIP of	US 1996-682853	19960712
		CIP of	US 1999-333145	19990614
			US 2001-777430	20010206
EP 1385996	A2		EP 2002-724912	20020206
			WO 2002-US3423	20020206

FILING DETAILS:

PATENT NO	KIND		PATENT NO
US 2002128465	A1	CIP of	US 6001567
EP 1385996	A2	Based on	WO 2002063030

PRIORITY APPLN. INFO: US 2001-777430 20010206; US 1996-682853 19960712; US 1999-333145 19990614
AN 2002-674850 [72] WPIDS
CR 1997-393613 [36]; 1998-322748 [28]; 1998-557036 [47]; 2002-083110 [11]; 2002-750464 [81]; 2003-596420 [56]; 2003-697784 [66]
AB WO 200263030 A UPAB: 20040210
NOVELTY - Composition comprises a positively or neutrally **charged** phosphoramidite.
DETAILED DESCRIPTION - Composition (c) or (c') comprises a positively

charged phosphoramidite of formula (I) or a neutrally **charged** phosphoramidite of formula (II). (I) comprises nitrogen-containing chemical group selected from primary, secondary or tertiary amine or ammonium group. (II) comprises secondary or tertiary amine or ammonium group.

X, Z = a reactive phosphate group;
Y = a protected hydroxy group;
X' = a protected hydroxy group;
N, N' = an amine group.

INDEPENDENT CLAIMS are included for the following:

(1) a composition (c1) comprising a **charge** tag (x1) attached to a terminal end of a nucleic acid molecule, the **charge** tag comprises a phosphate group and a positively **charged** molecule;

(2) a composition (c2) comprising a nucleic acid molecule that comprises a positively **charged** phosphoramidite;

(3) a composition (c3) comprising a **charge** tag attached to the terminal end of a nucleic acid molecule, the **charge** tag comprises a positively **charged** phosphoramidite;

(4) a composition (c4) comprising a fluorescent dye directly bonded to a phosphate group, which is not directly bonded to an amine group;

(5) a mixture (m) comprising a number of oligonucleotides, each oligonucleotide is attached to a different **charge** tag with each **charge** tag comprising a phosphate group and a positively **charged** group;

(6) a composition (c5) comprising a solid support attached to a **charged** tag, the **charge** tag comprises a positively **charged** group and a reactive group configured to allow the **charge** tag to covalently attach to the nucleic acid molecule;

(7) separating nucleic acid molecules involving either:

(a) treating (m1) a **charge**-balanced oligonucleotide containing the **charge** tag to produce a **charge**-unbalanced oligonucleotide and separating the **charge**-unbalanced oligonucleotide from the reaction mixture; or

(b) treating (m2) a number of **charge**-balanced oligonucleotides, each containing different **charge** tags, to produce at least 2 **charge**-unbalanced oligonucleotides, and separating the **charge**-unbalanced oligonucleotides from the reaction mixture.

USE - The composition is useful for separation of nucleic acid molecules (claimed). The composition is further useful for fractionation of specific nucleic acids by selective **charge** reversal useful in e.g. INVADER assay cleavage reactions; and in the synthesis of **charge**-balanced molecules.

ADVANTAGE - In the fractionation of nucleic acid molecules, the method provides an absolute readout of the partition of **products** from **substrates** (i.e. provides a 100% **separation**). Through the use of multiple positively **charged** adducts, synthetic molecules can be constructed with sufficient modification due to the fact that the normally negatively **charged** strand is made nearly neutral. It is also possible to distinguish between a enzymatically or thermally degraded DNA fragments due to the absence or presence of 3'phosphate.

Dwg.0/46

L19 ANSWER 9 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-316731 [36] WPIDS
DOC. NO. CPI: C2002-092216
TITLE: Measuring nitric oxide synthase activity, useful for

identifying therapeutic **enzyme** modulators,
using filter membrane to recover unconverted arginine substrate.

DERWENT CLASS: B04 D16
INVENTOR(S): HENNIES, H; SUNDERMANN, B
PATENT ASSIGNEE(S): (CHEF) GRUENENTHAL GMBH
COUNTRY COUNT: 98
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
DE 10043845	A1	20020314	(200236)*		15
WO 2002020831	A2	20020314	(200236)	GE	
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2001087711	A	20020322	(200251)		
EP 1315832	A2	20030604	(200337)	GE	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					
US 2004002129	A1	20040101	(200402)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
DE 10043845	A1		DE 2000-10043845	20000906
WO 2002020831	A2		WO 2001-EP10151	20010904
AU 2001087711	A		AU 2001-87711	20010904
EP 1315832	A2		EP 2001-967309	20010904
			WO 2001-EP10151	20010904
US 2004002129	A1	Cont of	WO 2001-EP10151	20010904
			US 2003-379717	20030306

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001087711	A	Based on	WO 2002020831
EP 1315832	A2	Based on	WO 2002020831

PRIORITY APPLN. INFO: DE 2000-10043845 20000906
AN 2002-316731 [36] WPIDS
AB DE 10043845 A UPAB: 20020610
NOVELTY - Measuring nitric oxide synthase (NOS) activity by:
(i) incubating NOS with labeled arginine (I) substrate;
(ii) separating (I) from labeled citrulline (II) formed as product, at a time when the (II) concentration is increasing, and
(iii) measuring amount of separated (I), the new feature is that separation is done using a filter-plate membrane (A).
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) identifying modulators of NOS, with addition of test compound in step (i); and
(2) use of **cation-exchange** (A) in methods where a NOS-mediated reaction occurs.

USE - The method is used to identify modulators, especially inhibitors, of NOS, potentially useful as therapeutic agents.

ADVANTAGE - Since the method provides very simple and quantitative **separation** of **substrate** and end **product**, it is suitable for high-throughput screening, and is so precise that multiple measurements are not necessary.

Dwg.0/1

L19 ANSWER 10 OF 66 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2002462402 MEDLINE
DOCUMENT NUMBER: 22209860 PubMed ID: 12220571
TITLE: Properties of glutaminase of crayfish CNS: implications for axon-glia signaling.
AUTHOR: Engler J A; Gottesman J M; Harkins J C; Urazaev A K; Lieberman E M; Grossfeld R M
CORPORATE SOURCE: Zoology Department and WM Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695-7617, USA.
CONTRACT NUMBER: 1 R01 NS34799-01A1 (NINDS)
SOURCE: NEUROSCIENCE, (2002) 114 (3) 699-705.
Journal code: 7605074. ISSN: 0306-4522.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020911
Last Updated on STN: 20021221
Entered Medline: 20021220

AB Glutaminase of crayfish axons is believed to participate in recycling of axon-glia signaling agent(s). We measured the activity and properties of glutaminase in crude homogenates of crayfish CNS, using **ion exchange** chromatography to **separate** radiolabeled **product** from **substrate**. Crayfish glutaminase activity is cytoplasmic and/or weakly bound to membranes and dependent on time, tissue protein, and glutamine concentration. It resembles the kidney-type phosphate-activated glutaminase of mammals in being stimulated by inorganic phosphate and alkaline pH and inhibited by the product glutamate and by the glutamine analog 6-diazo-5-oxo-L-norleucine. During incubation of crayfish CNS fibers in Na(+)-free saline containing radiolabeled glutamine, there is an increased formation of radiolabeled glutamate in axoplasm that is temporally associated with an increase in axonal pH from about 7.1 to about 8.0. Both the formation of glutamate and the change in pH are reduced by 6-diazo-5-oxo-L-norleucine. Our results suggest that crayfish glutaminase activity is regulated by cellular changes in pH and glutamate concentration. Such changes could impact availability of the axon-glia signaling agents glutamate and N-acetylaspartylglutamate.

L19 ANSWER 11 OF 66 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2002378040 MEDLINE
DOCUMENT NUMBER: 22119101 PubMed ID: 12123667
TITLE: Radiochemical malonyl-CoA decarboxylase assay: activity and subcellular distribution in heart and skeletal muscle.
AUTHOR: Kerner Janos; Hoppel Charles L
CORPORATE SOURCE: Department of Nutrition, Case Western Reserve University, Cleveland, Ohio 44106, USA.
CONTRACT NUMBER: P01 AG15885 (NIA)
SOURCE: ANALYTICAL BIOCHEMISTRY, (2002 Jul 15) 306 (2) 283-9.

Search completed by David Schreiber 308-4292

Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020719
Last Updated on STN: 20021217
Entered Medline: 20021210

AB Malonyl-CoA decarboxylase is the main route for the disposal of malonyl-CoA, the key metabolite in the regulation of mitochondrial fatty acid oxidation. We have developed a simple and sensitive radiochemical assay to determine malonyl-CoA decarboxylase activity. The decarboxylation of [2-14C]malonyl-CoA produces [2-14C]acetyl-CoA, which is converted to [2-14C]acetylcarnitine in the presence of excess L-carnitine and carnitine acetyltransferase. The positively **charged** radiolabeled **product**, acetylcarnitine, is **separated** from negatively **charged** excess radiolabeled **substrate** and the radioactivity measured by scintillation counting. Measurement of malonyl-CoA decarboxylase activities with this method gives values comparable to those obtained with assays currently in use, but has the advantage of being simpler and less labor intensive. We have applied this assay to rat skeletal muscle of different fiber-type composition and to rat heart. Malonyl-CoA decarboxylase activity (mU/g wet wt) correlates with the oxidative capacity of the muscles, being lowest in type IIb fibers (42.7 +/- 3.0) and highest in heart (1071.4 +/- 260), with intermediate activity in type IIa fibers (150.7 +/- 4.3) and type I fibers (107.8 +/- 7.6). Studies on subcellular distribution of malonyl-CoA decarboxylase activity in rat heart and rat skeletal muscle show that approximately 50 and 65% is localized to mitochondria, while 50 and 35% of the activity is extramitochondrial.

L19 ANSWER 12 OF 66 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:566316 SCISEARCH
THE GENUINE ARTICLE: 450TX
TITLE: A generic medium throughput activity assay procedure for serine-threonine protein phosphatases using C-14-labelled N-acetyl-Arg-Arg-Ala-Thr(P)-Val-Ala
AUTHOR: Sanvoisin J; Pollard J R; Hormozdiari P; Ward W H J; Gani D (Reprint)
CORPORATE SOURCE: Univ Birmingham, Sch Chem, Haworth Bldg, Birmingham B15 2TT, W Midlands, England (Reprint); Univ Birmingham, Sch Chem, Birmingham B15 2TT, W Midlands, England; AstraZeneca, Macclesfield SK10 4TG, Cheshire, England
COUNTRY OF AUTHOR: England
SOURCE: JOURNAL OF THE CHEMICAL SOCIETY-PERKIN TRANSACTIONS 1, (JUN 2001) No. 14, pp. 1709-1715.
Publisher: ROYAL SOC CHEMISTRY, THOMAS GRAHAM HOUSE, SCIENCE PARK, MILTON RD,, CAMBRIDGE CB4 0WF, CAMBS, ENGLAND.
ISSN: 1472-7781.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 46
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A new and sensitive activity assay for the Ser-Thr protein phosphatases PP1 and PP2A based upon the hydrolysis of the synthetic radiolabelled phosphopeptide substrate N-acetyl-Arg-Arg-Ala-Thr(P)-Val-Ala, is described. The protocol is also applicable to the assay of and PP2C

activity. The radiolabelled phosphopeptide is stable and can be stored for prolonged periods without deterioration or loss of radioactivity offering advantages over the use of P-32-labelled substrates. The assay method involves the **separation** of phosphopeptide **substrate** from the peptide alcohol **product** by **anion exchange** chromatography. The separation protocol is not sensitive to the presence of inorganic phosphate anion or metal cations. Using PP1 as the **enzyme** the radiochemical **assay** procedure afforded a V-max value of 17 (+/-2) muM s(-1) [28 (+/-3) muM s(-1) mug(-1)] and a K-M value of 3.7 (+/-0.9) mM for the substrate Ac-Arg-Arg-Ala-Thr(P)-Val-Ala with significantly greater accuracy than for a Malachite Green based assay. Inorganic phosphate was shown to be a competitive product inhibitor (K-i = 1.6 mM) and nodularin was found to be a potent competitive inhibitor (K-i = 0.19 nM) for PP1 with respect to the phosphopeptide substrate. This assay procedure was employed to determine the mode and magnitude of the inhibition of PP1 by the nodularin analogue described in the previous article [M. E. O'Donnell, J. Sanvoisin and D. Gani, J. Chemical Society, Perkin Trans. 1, 2001 (DOI:10.1039/b100402f)]. The **enzyme** PP2A afforded a V-max value of 2.8 (+/-0.2) muM s(-1) [45 (+/-2) muM s(-1)mug(-1)] and a K-M value of 4.1 (+/-0.7) mM for the radiolabelled substrate.

L19 ANSWER 13 OF 66 MEDLINE on STN DUPLICATE 5

ACCESSION NUMBER: 2001496948 MEDLINE
DOCUMENT NUMBER: 21425546 PubMed ID: 11545101
TITLE: Glucose biochip: dual analyte response in connection to two pre-column enzymatic reactions.
AUTHOR: Wang J; Chatrathi M P; Ibanez A
CORPORATE SOURCE: Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces 88003, USA.
CONTRACT NUMBER: RO1 RR14173-03 (NCRR)
SOURCE: ANALYST, (2001 Aug) 126 (8) 1203-6.
Journal code: 0372652. ISSN: 0003-2654.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20010910
Last Updated on STN: 20011001
Entered Medline: 20010927

AB This article describes a novel 'Lab-on-a-Chip' protocol generating two electrophoretic peaks for a single analyte, based on the coupling of two different pre-column enzymatic reactions of the same **substrate** followed by electrophoretic **separation** of the reaction **products**. Such operation is illustrated for the measurement of glucose in connection to the corresponding glucose oxidase (GOx) and glucose dehydrogenase (GDH) reactions. The pre-column enzymatic reactions generate hydrogen peroxide and NADH species, that are separated (based on their different **charges**) and detected at the end-column amperometric detector. The peak current ratio can be used for confirming the peak identity, estimating the peak purity, addressing co-migrating interferences, and deviations from linearity. A driving voltage of 2000 V results in peroxide and NADH migration times of 93 and 260 s, respectively. Factors influencing the unique dual glucose response are examined and optimized. The concept can be extended to different target analytes based on the coupling of two pre-column reactions with electrophoretic separation of the reaction products.

L19 ANSWER 14 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-558227 [51] WPIDS
CROSS REFERENCE: 2001-211224 [21]
DOC. NO. NON-CPI: N2000-413107
DOC. NO. CPI: C2000-166211
TITLE: High throughput mass spectrometry screening comprising growing a cell and purifying a non-column separated component from the cell by off-line parallel adjustment of growing conditions, useful for screening **enzyme** reactions.
DERWENT CLASS: A96 B04 D16 J04 S03
INVENTOR(S): CHEN, Y H; KREBBER, C; RAILLARD, S A
PATENT ASSIGNEE(S): (MAXY-N) MAXYGEN INC; (LIUL-I) LIU L
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000048004	A1	20000817	(200051)*	EN	58
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000034899	A	20000829	(200062)		
EP 1151306	A1	20011107	(200168)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
KR 2001102069	A	20011115	(200231)		
KR 2002022808	A	20020327	(200264)		
JP 2003524394	W	20030819	(200356)		77

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000048004	A1	WO 2000-US3686	20000211
AU 2000034899	A	AU 2000-34899	20000211
EP 1151306	A1	EP 2000-913451	20000211
		WO 2000-US3686	20000211
KR 2001102069	A	KR 2001-710179	20010811
KR 2002022808	A	KR 2002-701884	20020209
JP 2003524394	W	JP 2000-598862	20000211
		WO 2000-US3686	20000211

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000034899	A	Based on	WO 2000048004
EP 1151306	A1	Based on	WO 2000048004
JP 2003524394	W	Based on	WO 2000048004

PRIORITY APPLN. INFO: US 2000-502283 20000211; US 1999-119766P 19990211; US 1999-148848P 19990812; US 2000-637309 20000811
AN 2000-558227 [51] WPIDS
CR 2001-211224 [21]

Search completed by David Schreiber 308-4292

AB WO 200048004 A UPAB: 20030903
NOVELTY - A method (M1) for high throughput mass spectrometry screening, comprising growing a cell, purifying at least 1 non-column-separated component from the cell by off-line parallel adjustment of cell growing conditions, and performing flow-Injection analysis using electrospray tandem mass spectrometry and obtaining mass-to-**charge** ratio data, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method (M2) for monitoring at least 1 product or reactant by high throughput mass spectrometry comprising:

(a) providing a cell that has been transformed with a plasmid containing at least 1 member of a library of related gene sequences;

(b) growing a cell colony or culture from the cell;

(c) producing the product or reactant from the cell colony or culture in a biological matrix, thus producing a non-column-separated sample;

(d) purifying the non-column separated sample from the biological matrix, the purifying comprising an off-line parallel adjustment of the biological matrix used for producing the non-column separated sample; and

(e) monitoring the non-column separated sample by flow-injection analysis using electrospray tandem mass spectrometry;

(2) an apparatus for high throughput mass spectrometry screening comprising:

(a) a cell growth plate for growing cells and reacting an **enzyme**, substrate and product;

(b) an off-line parallel purification system coupled to or within the cell growth plate;

(c) an automatic sampler operably coupled to the off-line parallel purification system; and

(d) a mass spectrometer operably coupled to the automatic sampler comprising a sample handler that transports samples from the off-line purification system to the mass spectrometer for injection and analysis;

(3) a method (M3) for analyzing a number of components comprising:

(a) providing number of components that are tagged;

(b) binding the tagged components to a tag binding group on a solid support;

(c) reacting the tagged component with at least 1 reagent;

(d) removing the tagged components from the reaction mixture or washing the reaction mixture from the solid support; and

(e) analyzing the tagged components, reagents or products in a high throughput system.

USE - The high throughput mass spectrometry is useful for screening **enzyme** reactions and libraries of shuffled molecules, screening plasma, urine or cerebral spinal fluid for identification of metabolites that correlate with cancer susceptibility, event specific detection of exposure to toxins, monitoring the effects of drug trials, monitoring the effects of prescribed drug use, and creation of a metabolite encyclopedia that contains metabolite profiles of every cell in the human body. The high throughout mass spectrometry is also useful for identification of the gene pathways responsible for synthesis of commercially valuable plant products, such as drugs, and oils, and for the identification of the effects of gene transformation on metabolite phenotype, or for screening plants for the presence of desired natural products.

ADVANTAGE - The methods allow analysis of a large number of samples in a short space of time, i.e. at least 100 samples in a day, because an off-line purification system is used.

Dwg.0/4

L19 ANSWER 15 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

Search completed by David Schreiber 308-4292

Gitomer 09/888,008

ACCESSION NUMBER: 2000-465512 [40] WPIDS
DOC. NO. NON-CPI: N2000-347494
DOC. NO. CPI: C2000-140127
TITLE: New glutamine fructose-6-phosphate aminotransferase II **enzyme** nucleic acids and polypeptides used for the diagnosis of glucose intolerance, and insulin resistance in non-insulin dependent diabetes mellitus.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): BROSCHAT, K O; COUGHENOUR, M; **CURRIE, M G;** DOTSON, S B; KASAI, Y; **MOORE, W M**
PATENT ASSIGNEE(S): (SEAR) SEARLE & CO G D
COUNTRY COUNT: 84
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000037617	A1	20000629	(200040)*	EN	99

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW

AU 2000022163 A 20000712 (200048)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000037617	A1	WO 1999-US30943	19991222
AU 2000022163	A	AU 2000-22163	19991222

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000022163	A	Based on	WO 2000037617

PRIORITY APPLN. INFO: US 1998-113421P 19981222
AN 2000-465512 [40] WPIDS
AB WO 200037617 A UPAB: 20000823
NOVELTY - Nucleic acid (I) comprising 20-500 nucleotides of a 2049 (human), 2921 (mouse), or 2672 (rat) base pair sequence, encoding a glutamine: fructose-6-phosphate aminotransferase II (GFAT II) **enzyme**, their complement, a sequence hybridizing to them, or a sequence encoding at least 15-100 residues of one of three 682 residue amino acid sequences, is new. All sequences fully defined in the specification.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid hybridizing specifically to a molecule encoding GFAT II, or its complement, but not hybridizing to a molecule encoding GFAT, or its complement;

(2) a GFAT II nucleic acid molecule, comprising at least 20 contiguous nucleotides of the 2049 base pair sequence;

(3) a GFAT II protein (II), encoded by the 2049 base pair sequence, or having the 682 amino acid sequence;

(4) an antibody specific for (II);

(5) a transformed cell, having a nucleic acid, comprising a

structural nucleic acid encoding a GFAT II protein, peptide, or fragment;

(6) determining the level or pattern of GFAT II expression in a cell, comprising:

(a) incubating under hybridization conditions, a marker capable of specific hybridization to a nucleic acid encoding GFAT under high stringency conditions, with a nucleic acid derived from the cell;

(b) permitting hybridization between the marker and a complementary sequence in the cell nucleic acid; and

(c) detecting the level or pattern of hybridization;

(7) detecting the presence of a mutation affecting the level or pattern of GFAT II expression, comprising:

(a) incubating, under hybridization conditions, a marker comprising a nucleic acid linked to a gene specifically hybridizing to the 2049 base pair sequence, or its complement in nucleic acid from the cell, the hybridization permits detection of a polymorphism which predicts a mutation affecting the level or pattern of GFAT II protein in the cell;

(b) permitting hybridization; and

(c) detecting the presence of hybridization;

(8) determining an association between a polymorphism and a trait, comprising hybridizing the 2049 base pair sequence to genetic material in the cell, and calculating the degree of associating between the polymorphism and the trait;

(9) producing a cell overexpressing a GFAT II protein, comprising introducing into a cell a functional nucleic acid comprising the 2049 base pair sequence, and culturing the cell;

(10) producing a cell expressing reduced levels of a GFAT II protein, comprising introducing into a cell a functional nucleic acid comprising the 2049 base pair sequence, and a molecule causing co-suppression of the GFAT, and culturing the cell;

(11) reducing GFAT II protein expression in a cell, comprising introducing a nucleic acid having an exogenous promoter region linked to a transcribed nucleic acid whose transcribed strand is complementary to the 2049 base pair sequence, or its complement, and the non-transcribed strand is complementary to an endogenous mRNA, and culturing the cell;

(12) isolating a nucleic acid encoding GFAT II protein, comprising incubating under hybridization conditions a nucleic acid complementary to the 2049 base pair sequence, and a nucleic acid from the cell, permitting hybridization, and isolating the second nucleic acid;

(13) identifying a molecule, compound, or composition, effecting GFAT activity of GFAT II, comprising contacting a GFAT II with a test sample comprising the compound or molecule, and comparing the GFAT activity to a control;

(14) a nucleic acid encoding a mammalian GFAT II protein; and

(15) determining **enzyme activity** in an automated high-throughput format, comprising contacting an **enzyme** with its labeled **substrate** in a multi-well plate, **separating** the **substrate** from differently **charged product** using **ion exchange** resin, and detecting the amount of product or substrate bound to the resin.

ACTIVITY - None given.

MECHANISM OF ACTION - GFAT II modulators. No biological data is given.

USE - The methods can be used for diagnosis and prognosis of insulin resistance in non-insulin dependent diabetes mellitus, and comprise assaying the concentration of a molecule, preferably a protein, expressed by GFAT II gene, or mRNA or cDNA encoded by GFAT II gene, whose concentration is dependent on GFAT II gene expression, in cells or body fluid, such as skeletal tissue, blood, lymph or serum (claimed). The concentration is compared to a sample from a non-predisposed mammal

(claimed). The mRNA is assayed by incubating a sample of the body fluid in the presence of a nucleic acid hybridizing to the mRNA (claimed). The methods can also be used for diagnosis and prognosis of glucose intolerance or insulin resistant non-insulin dependent diabetes mellitus, comprising incubating under hybridization conditions, a marker specific for a polynucleotide linked to GFAT II gene, with a nucleic acid derived from a cell, or from within a body fluid, and detecting hybridization which detects a polymorphism predicting a mutation affecting GFAT II response, preferably GFAT II level, or pattern, in the patient (claimed). The marker comprises a nucleotide sequence physically linked to within 1 mbase, preferably 10 kbase of a sequence hybridizing to a GFAT II gene (claimed). Markers can be used to determine levels, or patterns, of GFAT II expression (claimed). GFAT II modulators can be identified (claimed). The modulators can be used in pharmaceutical compositions to treat conditions associated with abnormal GFAT II expression.

ADVANTAGE - None given.

Dwg.0/0

L19 ANSWER 16 OF 66 MEDLINE on STN DUPLICATE 6

ACCESSION NUMBER: 2000127798 MEDLINE
DOCUMENT NUMBER: 20127798 PubMed ID: 10660464
TITLE: Detection of phosphopeptides by fluorescence polarization in the presence of cationic polyamino acids: application to kinase assays.
AUTHOR: Coffin J; Latev M; Bi X; Nikiforov T T
CORPORATE SOURCE: Caliper Technologies Corp., 605 Fairchild Drive, Mountain View, California 94043, USA.
SOURCE: ANALYTICAL BIOCHEMISTRY, (2000 Feb 15) 278 (2) 206-12. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000330
Last Updated on STN: 20000330
Entered Medline: 20000322

AB We have studied the interaction of several phosphopeptides with cationic polyamino acids such as polyarginine and polylysine by fluorescence polarization. The phosphopeptides used were labeled with fluorescein, and their net **charges** at the experimental pH of 7. 5 were 0, -1, -2, and -3. These phosphopeptides represent the products of enzymatic phosphorylation reactions of the corresponding nonphosphorylated precursors by the protein kinase A, Akt1 (protein kinase Balpha), and protein kinase C. We found that these phosphopeptides bind more strongly to the cationic polyamino acids studied than their nonphosphorylated analogs. This preferential binding of the phosphorylated peptides could be conveniently detected by an increase in the fluorescence polarization signal of the attached fluorescein residue. We have exploited this observation to develop a new approach for the detection of kinase activity that does not require radioactivity or **separation** of **substrate** from **product**. We have successfully used this method to perform K(m) determinations of the kinase enzymes for their substrates and K(i) determinations of one of their inhibitors. This method for measuring kinase activity might be particularly useful for high-throughput screening applications.

L19 ANSWER 17 OF 66 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 2000269979 MEDLINE
DOCUMENT NUMBER: 20269979 PubMed ID: 10807973
TITLE: Characteristics of L-ornithine: 2-oxoacid aminotransferase and potential prenatal diagnosis of gyrate atrophy of the choroid and retina by first trimester chorionic villus sampling.
AUTHOR: Roschinger W; Endres W; Shin Y S
CORPORATE SOURCE: Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderspital, Lindwurmstrasse 4, D-80337, Munchen, Germany.. wulf.roeschinger@kk-i.med.uni-muenchen.de
SOURCE: CLINICA CHIMICA ACTA, (2000 Jun) 296 (1-2) 91-100. Journal code: 1302422. ISSN: 0009-8981.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20010529
Entered Medline: 20000714

AB A deficiency of the mitochondrial matrix **enzyme** L-ornithine: 2-oxoacid aminotransferase causes gyrate atrophy of the choroid and retina with hyperornithinemia (MIM 258870), a blinding degenerative disease, which is inherited as an autosomal recessive trait. We have developed a sensitive microradioisotopic method for **enzyme assay** by using 2-oxo-[5-14C] glutarate as the **substrate** and performing the **separation** of the **product**, [5-14C] glutamate from the substrate on a **cation-exchange** column. The **enzyme** activity was determined in human and rat tissues and in cultured cells. The **enzyme** activity in fibroblasts from a patient was deficient and that of the parents ranged between 25 and 60% of the control values. In addition we have found the **enzyme** expressed in native and cultured chorionic villi indicating a potential detection of the disease during the first trimester of pregnancy.

L19 ANSWER 18 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-136084 [12] WPIDS
CROSS REFERENCE: 1992-080072 [10]; 1996-361328 [36]; 1997-225429 [20]; 1998-494772 [42]; 1999-069659 [06]; 2000-037078 [03]; 2000-115881 [10]
DOC. NO. CPI: C2000-041603
TITLE: A biosensor comprising a crosslinked protein crystal is useful for detecting the presence of a substance in a sample.
DERWENT CLASS: A41 B04 D13 D15 D16 E16 J04
INVENTOR(S): NAVIA, M A; ST CLAIR, N L
PATENT ASSIGNEE(S): (VERT-N) VERTEX PHARM INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 6004768	A	19991221	(200012)*		50

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 6004768	A	CIP of	US 1990-562280	19900803

Search completed by David Schreiber 308-4292

CIP of	US 1991-720237	19910624
CIP of	US 1992-864424	19920406
Cont of	US 1993-17510	19930212
	US 1995-484238	19950607

FILING DETAILS:

PATENT NO	KIND		PATENT NO
US 6004768	A	Cont of	US 5618710

PRIORITY APPLN. INFO: US 1993-17510 19930212; US 1990-562280 19900803; US 1991-720237 19910624; US 1992-864424 19920406; US 1995-484238 19950607

AN 2000-136084 [12] WPIDS

CR 1992-080072 [10]; 1996-361328 [36]; 1997-225429 [20]; 1998-494772 [42]; 1999-069659 [06]; 2000-037078 [03]; 2000-115881 [10]

AB US 6004768 A UPAB: 20000308

NOVELTY - A biosensor for detecting an analyte of interest in a fluid comprises a protein crystal crosslinked with a multifunctional crosslinking agent, a retaining means and a signal transducer.

DETAILED DESCRIPTION - A biosensor for detecting an analyte of interest in a fluid comprises:

(1) a protein crystal crosslinked with a multifunctional crosslinking agent which has resistance to exogenous proteolysis so that the crosslinked protein crystal retains at least 91% of its stability, measured in terms of its degradation after incubation for 3 hours in the presence of a concentration of Pronase(TM) that causes the soluble crosslinked form of the protein to lose at least 94% of its stability, measured in terms of degradation under the same conditions where the protein has the activity of acting on the analyte of interest or on a reactant in a reaction which the analyte of interest participates;

(2) a retaining means for the crosslinked protein crystal consisting of a material which allows contact between the crosslinked protein crystal and a fluid which contains either the analyte on which the protein acts or a reactant in a reaction in which the analyte participates; and

(3) a signal transducer which produces a signal in the presence of the analyte.

An INDEPENDENT CLAIM is also included for an extracorporeal device which is used for altering a component of a fluid comprising a protein crystal, retaining means and signal transducer as above.

USE - The extracorporeal device is used for altering heparin, methotrexate, bilirubin, amino acids, urea or ammonia levels in a fluid.

The biosensor is used to detect the presence of a substance in a sample (claimed) and to remove substances from a sample. The sample can be a biological sample, water or other sample. It can also be used to catalyze the production of a selected product by altering a single substrate or combining the substrate with an additional substance or substances.

ADVANTAGE - The crosslinked enzyme crystals do not require a **separate**, inert support structure so **substrate** and **product** diffusion properties are improved and enzyme concentrations are provided which are close to the theoretical packing limit for the molecules, giving increased effective activity, reduction in substrate contact time with enzymes and reductions in plant size and capital costs. The enzyme can be used in harsh conditions e.g. elevated temperature, aqueous, organic or near-anhydrous solvents which was not possible with conventional immobilized enzyme systems.

Dwg.0/19

Search completed by David Schreiber 308-4292

L19 ANSWER 19 OF 66 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1999107568 MEDLINE
DOCUMENT NUMBER: 99107568 PubMed ID: 9888975
TITLE: A high-throughput radiometric assay for hepatitis C virus NS3 protease.
AUTHOR: Cerretani M; Di Renzo L; Serafini S; Vitelli A; Gennari N; Bianchi E; Pessi A; Urbani A; Colloca S; De Francesco R; Steinkuhler C; Altamura S
CORPORATE SOURCE: Istituto di Ricerche di Biologia Molecolare (IRBM) "P. Angeletti,", Pomezia, Rome, 00040, Italy.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Jan 15) 266 (2) 192-7. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990311
Last Updated on STN: 20000303
Entered Medline: 19990224

AB A novel radiometric in vitro assay for discovery of inhibitors of hepatitis C viral protease activity, suitable for high-throughput screening, was developed. The NS3 protein of hepatitis C virus (HCV) contains a serine protease, whose function is to process the majority of the nonstructural proteins of the viral polyprotein. The viral NS4A protein is a cofactor of NS3 protease activity in the cleavage of NS3-NS4A, NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B junctions. To establish an in vitro assay system we used NS3 proteases from different HCV strains, purified from Escherichia coli and a synthetic radiolabeled peptide substrate that mimics the NS4A-NS4B junction. Upon incubation with the **enzyme** the **substrate** was **separated** from the radiolabeled cleavage **product** by addition of an **ion exchange** resin. The assay was performed in a microtiter plate format and offered the potential for assaying numerous samples using a laboratory robot. Taking advantage of these features, we used the assay to optimize reaction conditions by simultaneously varying different buffer components. We showed that physicochemical conditions affect NS3 protease activity in a strain-specific way. Furthermore, the sensitivity of the assay makes it suitable for detection and detailed mechanistic characterization of inhibitors with low-nanomolar affinities for the HCV serine protease.

L19 ANSWER 20 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 9
ACCESSION NUMBER: 1998:270109 BIOSIS
DOCUMENT NUMBER: PREV199800270109
TITLE: Purification of swine serum angiotensin converting **enzyme** with high recovery of activity using lisinopril coupled to epoxy-activated sepharose 6B.
AUTHOR(S): Quassinti, Luana; Miano, Antonino; Bramucci, Massimo; Maccari, Ennio; Amici, Domenico [Reprint author]
CORPORATE SOURCE: Dep. Mol. Cell. Anim. Biol., Univ. Camerino, Camerini n.2, I-62032 Camerino, MC, Italy
SOURCE: Biochemistry and Molecular Biology International, (April, 1998) Vol. 44, No. 5, pp. 887-895. print. ISSN: 1039-9712.
DOCUMENT TYPE: Article

Search completed by David Schreiber 308-4292

LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jun 1998
Last Updated on STN: 13 Aug 1998

AB The Authors describe the purification of swine serum ACE to molecular homogeneity with high recovery of activity (40%) in a few steps. The purification procedure consists of affinity chromatography, using commercial activated resin (epoxy-activated sepharose 6B) and two steps of **anion exchange** chromatography (Resource Q) performed at different pH (pH 9.0 and pH 6.0). Furthermore, a specific and sensitive method for the accurate quantitation of ACE activity in biological fluids was developed, based on the hydrolysis of synthetic FAPGG (N-(3-(2-furyl) acryloyl) L-phenylalanyl glycyl glycine), as **substrate** and following the **separation** of **products** by reversed-phase HPLC. Some kinetic parameters were determined. The Km and Kcat values for FAPGG were 0.793 +- 0.052 mM and 5830 s-1, respectively, and the 150 values for captopril and lisinopril, two specific ACE inhibitors, are 5.7 +- 0.67 nM and 1.0 +- 0.29 nM, respectively.

L19 ANSWER 21 OF 66 MEDLINE on STN
ACCESSION NUMBER: 1998110303 MEDLINE
DOCUMENT NUMBER: 98110303 PubMed ID: 9448836
TITLE: Measurement of alpha(1-3) fucosyltransferase activity using scintillation proximity.
AUTHOR: Hood C M; Kelly V A; Bird M I; Britten C J
CORPORATE SOURCE: Glycobiology Research Unit, Glaxo Wellcome Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1998 Jan 1) 255 (1) 8-12.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980410
Last Updated on STN: 19980410
Entered Medline: 19980327

AB The alpha 3 fucosyltransferases are a family of glycosyltransferases involved in the addition of fucose onto glycoproteins and glycolipids. One of the best defined roles for the alpha 3 fucosyltransferases is in the biosynthesis of the carbohydrate antigen sialyl Lewis X, the minimal ligand for the selectin family of adhesion molecules. We describe here the development of a single-step assay for the measurement of alpha 3 fucosyltransferase activity based on the principle of scintillation proximity. The fucosyltransferase catalyses the transfer of [3H]fucose, from GDP-[3H]fucose, onto the sugar chains of a glycoprotein acceptor noncovalently bound to a scintillant-impregnated microsphere (SPA bead). The resultant signal can be used as a **measure** of **enzyme** activity. Due to the nature of this assay no steps are required to **separate** unused **substrate** from **product**.
Kinetic data from the assay compare favorably with those obtained from assays currently used for the alpha 3 fucosyltransferases. This SPA-based assay appears generic for the alpha 3 fucosyltransferases and readily adaptable for other glycosyltransferases. The particular advantage of the assay is anticipated to be found in the simple, routine testing of a large number of samples.

L19 ANSWER 22 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 1997-272133 [24] WPIDS
CROSS REFERENCE: 1996-010559 [01]; 1998-008050 [01]

Search completed by David Schreiber 308-4292

Gitomer 09/888,008

DOC. NO. NON-CPI: N1997-225454
DOC. NO. CPI: C1997-087595
TITLE: Lawn assays, useful for screening libraries - for identifying compounds which affect enzymatic reaction or which bind to target molecules.
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): BURBAUM, J J; CHELSKY, D
PATENT ASSIGNEE(S): (PHAR-N) PHARMACOPEIA INC
COUNTRY COUNT: 74
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9716569	A1	19970509	(199724)*	EN	39
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN					
AU 9675535	A	19970522	(199739)		
US 5856083	A	19990105	(199909)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 9716569	A1		WO 1996-US17702	19961024
AU 9675535	A		AU 1996-75535	19961024
US 5856083	A	CIP of	US 1994-239302	19940506
		CIP of	US 1995-436120	19950508
			US 1995-553056	19951103

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9675535	A	Based on	WO 9716569

PRIORITY APPLN. INFO: US 1995-553056 19951103; US 1994-239302 19940506; US 1995-436120 19950508
AN 1997-272133 [24] WPIDS
CR 1996-010559 [01]; 1998-008050 [01]
AB WO 9716569 A UPAB: 19990302
Lawn assay for identifying compounds which affect an enzymatic reaction or which bind to a target molecule comprises: (1) (a) providing an enzyme (EZ) or target molecule (TM); (b) where EZ is provided further providing a substrate for EZ and where TM is provided further providing a labelled ligand bound to TM; (c) providing supports having multiple copies attached to them via cleavable linkers of a compound to be screened for its effect on the enzymatic reaction or its ability to bind to the target molecule; (d) cleaving the compounds from the supports; (e) contacting the supports with a colloidal matrix (CM) so that the compounds diffuse into the CM; (f) carrying out the enzymatic reaction or the binding of compounds to TM in the CM; and (g) monitoring a photometrically detectable change in: (i) the substrate or a coenzyme or enzyme cofactor involved in the enzymatic reaction; or (ii) labelled ligand, to determine a zone of activity in the matrix associated with one or more of the compounds. Also claimed are lawn assays comprising: (2) (a) as (1) (a)-(c) above; (b) contacting the supports with CM and cleaving the compounds from the supports either

before or after the contacting, so that the compounds diffuse into the matrix; and (c) as (1) (f) and (g) above, to determine a zone of activity in the matrix associated with one or more of the compounds where the cleavable linker is not photocleavable; (3) (a) providing a CM containing a receptor having a ligand bound to it, which when bound to the receptor, the ligand emits a different photometrically detectable signal which indicates receptor binding, the CM further comprising solid supports, each of which has multiple copies attached via a cleavable linker of a compound to be screened for its ability to bind to the receptor; (b) as (1) (d) above; and (c) detecting the difference to localise one or more of the compounds which bind to the receptor; (4) (a) providing EZ and a substrate for EZ, selected such that the substrate converts to a product having a different net **charge** as a result of the enzymatic reaction; (b) providing solid supports, each having multiple copies attached via a cleavable linker of a compound to be screened for its effect on the enzymatic reaction; (c) contacting the solid supports with CM and cleaving the compounds from the supports, either before or after the contacting so that the compounds diffuse into the matrix; (d) as (1) (f) above; (e) electrophoretically **separating** the **substrate** and the **product**; and (f) detecting the substrate or the product to determine a zone of activity associated with one or more of the compounds; and (5) (a) providing TM; (b) providing a labelled ligand bound to TM; (c) as (1) (c) above; (d) as (2) (d) as above; (e) binding compounds to TM in CM; (f) monitoring photometrically detectable change in the labelled ligand, to determine a zone of activity in the matrix associated with one or more of the compounds.

USE - The methods are used to screen many compounds for their effect on EZ activity or their ability to bind to TM. The methods are used e.g. for screening proteases or enzyme inhibitors.

Dwg.0/4

L19 ANSWER 23 OF 66 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 1997-237517 [22] WPIDS
DOC. NO. CPI: C1997-076418
TITLE: Production of ultra-thin film reactor with immobilised proteins - by immersing solid carrier in aqueous protein solution and aqueous poly ion solution alternately.
DERWENT CLASS: B04 D16
INVENTOR(S): ARIGA, K; KUNITAKE, T; LVOV, Y; ONDA, M
PATENT ASSIGNEE(S): (NISC-N) JAPAN SCI & TECHNOLOGY CORP; (MITA) MITSUI CHEM INC; (ONDA-I) ONDA M; (SHKJ) SHINGIJUTSU JIGYODAN
COUNTRY COUNT: 5
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
DE 19642882	A1	19970424	(199722)*		15
AU 9668157	A	19970424	(199725)		
JP 09107952	A	19970428	(199727)		9
JP 2909959	B2	19990623	(199930)		9
AU 709355	B	19990826	(199946)		
US 6107084	A	20000822	(200042)		
CN 1163311	A	19971029	(200318)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
DE 19642882	A1	DE 1996-19642882	19961017

Search completed by David Schreiber 308-4292

AU 9668157	A	AU 1996-68157	19961014
JP 09107952	A	JP 1995-270814	19951019
JP 2909959	B2	JP 1995-270814	19951019
AU 709355	B	AU 1996-68157	19961014
US 6107084	A	US 1996-730929	19961016
CN 1163311	A	CN 1996-117293	19961019

FILING DETAILS:

PATENT NO	KIND		PATENT NO
JP 2909959	B2	Previous Publ.	JP 09107952
AU 709355	B	Previous Publ.	AU 9668157

PRIORITY APPLN. INFO: JP 1995-270814 19951019
AN 1997-237517 [22] WPIDS
AB DE 19642882 A UPAB: 19970530
Production (A) of an ultra-thin film reaction with immobilised proteins comprises immersing a solid carrier in an aqueous solution and an aqueous solution of a polyion, alternately.

The polyion **charge** is opposite to the **charge** on the protein and the product has a structurally controlled ultra-thin film with molecular precision content, on the carrier.

Also claimed are:

(1) a production (B) of an ultra-thin film reactor supplying at least 2 proteins, by:

(a) immersing a solid carrier with an electrically **charged** surface in an aqueous solution which contains polyions or protein with the opposite **charge**, to reverse the **charge** on the surface by neutralisation and re-saturation,

(b) immersing in a different aqueous solution containing polyions or protein of the opposite **charge**, to reverse the **charge** again, by neutralisation and re-saturation and

(c) repeating the steps;

(2) a process for chemically reacting substrate molecules, using a carrier with several immobilised thin films, obtained by (A) or (B), and

(3) a process for chemically reacting substrate molecules by immobilising a thin film of protein on a solid carrier which has a separation function, obtained by (A) or (B), and **separating** the **substrate** and the **product** of the reaction.

USE - The processes are used for reactions with immobilised enzymes (claimed). The reactor may be created with a set of immobilised enzymes that mimics an in vivo system or the reactor may be part of a sensor system.

ADVANTAGE - The process is simple and versatile. As only electrostatic forces are used to bind the protein, the protein structure is not altered.

The polyion is flexible, so that substrate can easily penetrate the structure. The carrier may be a porous material.

Dwg.1/9

L19 ANSWER 24 OF 66 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1998121351 MEDLINE
DOCUMENT NUMBER: 98121351 PubMed ID: 9460049
TITLE: Protein architecture: assembly of ordered films by means of alternated adsorption of oppositely **charged** macromolecules.
AUTHOR: Lvov YuM; Sukhorukov G B
CORPORATE SOURCE: Institute of Crystallography, Russian Academy of Sciences,

Moscow.
SOURCE: MEMBRANE AND CELL BIOLOGY, (1997) 11 (3) 277-303. Ref: 88
Journal code: 9517472. ISSN: 1023-6597.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980326
Last Updated on STN: 19980326
Entered Medline: 19980317

AB Methods of making molecularly ordered protein films are reviewed with special reference to the recently developed technique of protein multilayer assembly by alternated adsorption of opposite-**charge** polyions. This method has been applied for linear and branched polyions, DNA, polynucleotides, proteins, viruses and clay nanoplates. This provides good prospects for biomolecular architecture. Quartz crystal microbalance, X-ray and neutron reflectivity, scanning electron microscopy, atomic force microscopy and UV-absorbance data are used to analyze the film structure. Multilayer buildup by alternation of polyions and 16 different **charged** proteins is discussed. In most cases, enzymes in the films retained their activity. Protein/ceramic nanoplates consisting of alternated montmorillonite clay and glucose oxidase layers electrostatically linked by polycations were also assembled. Protein layers can be arranged according to specific biological activity. Consecutive enzymic reactions were performed in anisotropic protein layers prepared with precise control of distances between the active layers (1-50 nm). Film superlattices containing ordered layers of more than one protein were constructed using myoglobin, lysozyme, peroxidase, glucoamylase, glucose oxidase and catalase. Glucoamylase, glucose oxidase/peroxidase catalyze the starch-glucose-H2020 reaction. The reaction **products** and nonreacting starch were **separated** by filtration when the **substrate** solution passed through the multienzyme films assembled on a filter. Formation of alternate outermost layers (of opposite **charge** or opposite specificity) at every adsorption cycle is the key point of the layer-by-layer assembly. Multilayers were obtained by alternated adsorption of concanavalin A and glycogen (or streptavidin and biotinylated polylysine) were designed using their biospecific interaction. Protein films are of extreme interest as novel biologically active materials.

L19 ANSWER 25 OF 66 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

ACCESSION NUMBER: 97:567001 SCISEARCH
THE GENUINE ARTICLE: XK380
TITLE: Protein architecture: Assembly of ordered films by means alternated adsorption of opposite **charged** macromolecules
AUTHOR: Lvov Y M (Reprint); Sukhorukov G B
CORPORATE SOURCE: RUSSIAN ACAD SCI, INST CRYSTALLOG, LENINSKY PR 59, MOSCOW 117333, RUSSIA (Reprint)
COUNTRY OF AUTHOR: RUSSIA
SOURCE: BIOLOGICHESKIE MEMBRANY, (MAY-JUN 1997) Vol. 14, No. 3, pp. 229-250.
Publisher: MEZHDUNARODNAYA KNIGA, 39 DIMITROVA UL., 113095 MOSCOW, RUSSIA.
ISSN: 0233-4755.
DOCUMENT TYPE: General Review; Journal

FILE SEGMENT: LIFE
LANGUAGE: Russian
REFERENCE COUNT: 88
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Methods for creation of molecularly ordered protein films are reviewed with a special attention to the recently developed technique of protein multilayer assembly by alternate adsorption of opposite **charged** polyions. This method has been applied for linear and branced polyions, DNA, polynucleotides, proteins, viruses and clay nanoplates. That provides good prospects for biomolecular architecture. Quartz crystal microbalance, X-ray and neutron reflectivity, scanning electron microscopy, AFM and UV-absorbance data are used for analysis of the film structure. Multilayer buildup by alternation of polyions and 16 different **charged** proteins is discussied. In most cases, enzymes in the films retained their activity. Protein/ceramic nanocomposites consisting of alternating montmorillonite clay and glucose oxidase layers electrostatically connected through polycations were also assembled.

Protein layers may be arranged according to specific biological activity. Sequential enzyme reactions were performed by preparation of anisotropic protein layers and precise control of distances between active layers (1-50 nm). Film superlattices containing ordered layers of more than one protein were constructed with myoglobin, lysozyme, peroxidase, glucoamylase, glucose oxidane and catalase. Glucoamylase glucose oxidase/peroxidase catalyse the reaction starch-glucose-H2O2. The reaction **products** and nonreacted starch were **separated** by filtration when the **substrate** solution passed these multienzyme films assembled on a filter.

The formation of alternate outermost layers (the opposite **charge** or the opposite specificity) at every adsorption cycle is the key point of the layer-by-layer assembly. Thus, multiyaers were obtained by alternate adsorption of concanavalin A and glycogen (or streptavidin and biotinylated polylysine) were designed using their biospecific interaction. Protein films are extremely interesting as novel biologically active materials.

L19 ANSWER 26 OF 66 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 97115656 MEDLINE
DOCUMENT NUMBER: 97115656 PubMed ID: 8954971
TITLE: A comparative analysis of the primary sequences and characteristics of heparinases I, II, and III from Flavobacterium heparinum.
AUTHOR: Godavarti R; Sasisekharan R
CORPORATE SOURCE: Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, 02139, USA.
CONTRACT NUMBER: DK 51207 (NIDDK)
GM 31318 (NIGMS)
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1996 Dec 24) 229 (3) 770-7.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19980206
Entered Medline: 19970130

AB Heparinases I, II and III from F. heparinum cleave heparin-like molecules, with a high degree of substrate specificity, at the glucosamine-uronate

linkage by elimination, leaving an unsaturated C4-C5 bond in the uronic acid. The primary sequences of these **enzymes** have been reported earlier. In this study we perform a comparative analysis of the properties and primary sequences of heparinase I, II and III. Alignment of the primary sequences revealed little sequence homology (15% residue identity in a LALIGN alignment) at both DNA and amino acid levels. There are three basic clusters in heparinase II satisfying the heparin binding consensus sequence with one of the sequences sharing homology with a consensus sequence in the heparin binding site of heparinase I and two basic clusters in heparinase III. Similar to heparinase I, there are two putative 'EF-hand' calcium coordinating motifs in heparinase III, while heparinase II does not contain any such motifs. Recombinant heparinases II and III's degradation of the **substrate** and the subsequent **separation** of the oligosaccharide **products** by POROS **anion exchange** chromatography were identical to those obtained from native heparinases II and III from F. heparinum.

L19 ANSWER 27 OF 66 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 96117318 MEDLINE
DOCUMENT NUMBER: 96117318 PubMed ID: 8527866
TITLE: Screening of colon tumor cells and tissues for folylpolyglutamate synthetase activity.
AUTHOR: van der Wilt C L; Cloos J; de Jong M; Pinedo H M; Peters G J
CORPORATE SOURCE: Department of Medical Oncology, Free University Hospital, Amsterdam, The Netherlands.
SOURCE: ONCOLOGY RESEARCH, (1995) 7 (6) 317-21.
Journal code: 9208097. ISSN: 0965-0407.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 19960220
Last Updated on STN: 19980206
Entered Medline: 19960201

AB Polyglutamylation of (anti)folates catalyzed by folylpolyglutamate synthetase (FPGS) determines the retention of these compounds in the cell. This feature is essential for the activity of folates (e.g., folinic acid) and antifolates (e.g., methotrexate) in the treatment of cancer. A FPGS assay was developed using murine liver and was based on published methods, but had a novel analytic procedure. Tritiated glutamate and aminopterin served as substrates for FPGS, and after the reaction the mixture of **substrates** and **products** was **separated** by thin-layer chromatography. Results were verified by standard **anion-exchange** high performance liquid chromatography for folates. The assay was applied to measure the activity of FPGS in several cancer cell lines and human and murine (tumor) tissues. Cancer cell lines had a much higher activity (varying from 82 to 656 pmol diglutamate formed per hour per 10(6) cells) than murine bone marrow cells (35 pmol/h/10(6) cells). Murine gut mucosa had a very low FPGS activity compared to murine liver (7 vs. 24 pmol diglutamate/h/g wet weight), but the activity in murine colon tumors was comparable to or higher than that in liver (28-52 pmol diglutamate/h/g wet weight). A screening of 11 human colon tumors or metastases demonstrated that there was a large variation in FPGS activity in this tumor type, but overall the activity was higher in tumor tissue than in normal colon mucosa. The latter feature may increase the selectivity of antifolate-based chemotherapy of colon tumors. The FPGS assay described in this paper allows large-scale screening of

cell lines and tissues, because of its rapid separation procedure by thin-layer chromatography.

L19 ANSWER 28 OF 66 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 94315989 MEDLINE
DOCUMENT NUMBER: 94315989 PubMed ID: 7518887
TITLE: Nitric oxide participates in the regulation of pancreatic acinar cell secretion.
AUTHOR: Wrenn R W; **Currie M G**; Herman L E
CORPORATE SOURCE: Department of Cellular Biology and Anatomy, School of Medicine, Medical College of Georgia, Augusta 30912.
SOURCE: LIFE SCIENCES, (1994) 55 (7) 511-8.
Journal code: 0375521. ISSN: 0024-3205.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940905
Last Updated on STN: 19960129
Entered Medline: 19940825

AB The role of nitric oxide (NO) in the regulation of exocrine secretion was investigated in isolated rat pancreatic acini. NO synthase activity was detected in the extract of acini and purified by **ion-exchange** and 2',5'-ADP agarose chromatographies. **Enzyme activity** was determined by conversion of 3H-arginine to 3H-citrulline, by measurement of nitrite (a breakdown product of NO) and by generation of cyclic GMP. Treatment of acini with L-arginine increased nitrite as well as cyclic GMP and amylase release, which were prevented by the nitric oxide synthase inhibitors N-monomethyl-arginine [NMMA] and NG-nitro-L-arginine [NNA]. These nitric oxide inhibitors also blocked carbachol-induced amylase release as well as elevation of acinar cell cyclic GMP. NNA was a potent inhibitor of carbamylcholine-induced amylase release (estimate Ki = 2.2 uM). Nitric oxide apparently participates significantly in the overall control of pancreatic acinar cell secretory function.

L19 ANSWER 29 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 14
ACCESSION NUMBER: 1994:552076 BIOSIS
DOCUMENT NUMBER: PREV199598011624
TITLE: **Assays** for three **enzymes** involved in mevalonic acid metabolism.
AUTHOR(S): Sandmann, Gerhard; Albrecht, Manuela
CORPORATE SOURCE: Fakultaet Biologie, Univ. Konstanz, P.O. Box 5560, D-78434 Konstanz, Germany
SOURCE: Physiologia Plantarum, (1994) Vol. 92, No. 2, pp. 297-301.
CODEN: PHPLAI. ISSN: 0031-9317.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Dec 1994
Last Updated on STN: 23 Feb 1995

AB **Enzyme assays** have been developed for mevalonate (MVA) kinase, mevalonate phosphate (MVAP) kinase and mevalonate pyrophosphate (MVAPP) anhydrodecarboxylase. The procedures involve radioactively labelled **substrates** and **separation** of the reaction **products** by **anion exchange** chromatography. The separation on Dowex 1-X2 in self-packed microcolumns is simple, inexpensive and results in good separation of the MVA derivatives from

each other. Because separation of MVAPP from isopentenyl pyrophosphate (IPP) was not possible directly, samples or column fractions containing MVAPP and IPP simultaneously were dephosphorylated by alkaline phosphatase. The resulting MVA and isopentenol are then easily separated in the same system. The **assays** for all three **enzymes** not only allows the determination of activities in crude **enzyme** preparations but is also applicable to the in vitro determination of intermediate pools in the reaction sequence from MVA to IPP after using 14C-MVA as substrate. The major advantage is accuracy and ease of use. The utility of the methods was demonstrated for **enzyme** extracts from the higher plants Chenopodium and spinach as well as for the fungus Phycomyces.

L19 ANSWER 30 OF 66 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 95162290 MEDLINE
DOCUMENT NUMBER: 95162290 PubMed ID: 7858722
TITLE: Measurement of nucleoside diphosphate kinase-Nm23 activity by **anion-exchange** high-performance liquid chromatography.
AUTHOR: Pulido-Cejudo G; Gagnon J; Leclerc J M; Jamison K; Gordon J; Campione-Piccardo J
CORPORATE SOURCE: National Laboratory for Viral Oncology, LCDC, Health Canada, Ottawa, Ontario.
SOURCE: JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL APPLICATIONS, (1994 Oct 3) 660 (1) 37-47.
Journal code: 9421796. ISSN: 0378-4347.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950404
Last Updated on STN: 19970203
Entered Medline: 19950322

AB A first-order assay to detect the activity of nucleoside diphosphate kinase (NDP-kinase; EC 2.7.4.6) was developed. In this assay, the activity of NDP-kinase is measured using various deoxy- and ribonucleotide triphosphates as phosphate donors and dADP as phosphate acceptor. The **enzyme** activity is determined by quantifying, after **anion -exchange** HPLC, the amount of newly synthesized dATP. Contrary to the most common coupled **enzymic assays** or isotopic **assays** the use of different donor-acceptor pairs is not restricted. The resolution of the procedure described is limited only by the chromatographic **separation** of **substrate** and **product** pairs participating in the reaction.

L19 ANSWER 31 OF 66 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 93256286 MEDLINE
DOCUMENT NUMBER: 93256286 PubMed ID: 8387731
TITLE: An assay method for DNA topoisomerase activity based on separation of relaxed DNA from supercoiled DNA using high-performance liquid chromatography.
AUTHOR: Onishi Y; Azuma Y; Kizaki H
CORPORATE SOURCE: Department of Biochemistry, Tokyo Dental College, Mihama-ku, Japan.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 Apr) 210 (1) 63-8.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

Search completed by David Schreiber 308-4292

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930618
Last Updated on STN: 19970203
Entered Medline: 19930610

AB A method for assaying the activities of DNA topoisomerases I and II has been developed. The **assay** for type I and II **enzymes** is based on monitoring relaxation of supercoiled plasmid DNA in the absence or presence of ATP, respectively. The reaction **product**, relaxed DNA, was **separated** from the **substrate**, supercoiled pBR329 plasmid, by a linear gradient of NaCl using high-performance **ion-exchange** chromatography with a DEAENPR column. In this method, nanogram amounts of relaxed DNA are detectable within 30 min, indicating that a subtle change in the activity in the cells can be assayed precisely.

L19 ANSWER 32 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 17
ACCESSION NUMBER: 1992:91823 BIOSIS
DOCUMENT NUMBER: PREV199293048373; BA93:48373
TITLE: A TWO-STEP **ENZYMATIC** SYNTHESIS OF DIPEPTIDES.
AUTHOR(S): SCHWARZ A [Reprint author]; WANDREY C; STEINKE D; KULA M R
CORPORATE SOURCE: INST BIOTECHNOL, RES CENT JUELICH, D-5170 JUELICH, GERMANY
SOURCE: Biotechnology and Bioengineering, (1992) Vol. 39, No. 2, pp. 132-140.
CODEN: BIBIAU. ISSN: 0006-3592.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 12 Feb 1992
Last Updated on STN: 14 Apr 1992

AB A simple system is introduced to produce dipeptides continuously by **enzyme** catalyzed condensation of amino acid esters and amino acids amides. Synthesis of N-terminal free dipeptide-amides is achieved by means of carboxypeptidase Y. The peptide-amide is deamidated utilizing a newly isolated peptide-amidase. **Separation** of **substrates** and **products** is accomplished by **anion -exchange** chromatography. Modeling of the reactions shows that the two reactions have to be carried out in a cascade of two reactors in order to prevent hydrolysis of the peptide by the carboxypeptidase. Continuous production of Kyotorphin (H-TyArg-OH) with a space-time yield of 257 g/L · d shows the fesibility of this concept.

L19 ANSWER 33 OF 66 MEDLINE on STN DUPLICATE 18
ACCESSION NUMBER: 93089493 MEDLINE
DOCUMENT NUMBER: 93089493 PubMed ID: 1333733
TITLE: Direct assay method for guanosine 5'-monophosphate reductase activity.
AUTHOR: Nakamura H; Natsumeda Y; Nagai M; Shiotani T; Weber G
CORPORATE SOURCE: Laboratory for Experimental Oncology, Indiana University School of Medicine, Indianapolis 46202-5200.
CONTRACT NUMBER: CA-42510 (NCI)
SOURCE: ANALYTICAL BIOCHEMISTRY, (1992 Oct) 206 (1) 115-8.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

Search completed by David Schreiber 308-4292

ENTRY MONTH: 199301
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 19970203
Entered Medline: 19930107

AB A sensitive and simple micromethod for the accurate measurement of GMP reductase (EC 1.6.6.8) activity in crude extracts is described. The reaction **product** of [8-14C]IMP was **separated** from the **substrate** [8-14C]GMP by descending chromatography on Whatman DE81 **ion-exchange** paper. This separation method provides an analysis of the possible interfering reactions, such as the metabolic conversion of the substrate GMP to GDP, GTP, and/or guanosine, and guanine and the loss of the product IMP to inosine, hypoxanthine, and other metabolites. Low blank values (70-90 cpm) were obtained consistently with this assay because the IMP spot moves faster than the GMP spot. The major advantages of this method are direct measurement of GMP reductase activity in crude extracts, high sensitivity (with a limit of detection of < 10 pmol of IMP production), high reproducibility (< +/- 5%), and capability to measure activity in small samples (9 micrograms protein).

L19 ANSWER 34 OF 66 MEDLINE on STN DUPLICATE 19
ACCESSION NUMBER: 92042513 MEDLINE
DOCUMENT NUMBER: 92042513 PubMed ID: 1939478
TITLE: High-performance liquid chromatographic determination of histamine N-methyltransferase activity.
AUTHOR: Fukuda H; Yamatodani A; Imamura I; Maeyama K; Watanabe T; Wada H
CORPORATE SOURCE: Department of Pharmacology II, Osaka University, Faculty of Medicine, Japan.
SOURCE: JOURNAL OF CHROMATOGRAPHY, (1991 Jul 5) 567 (2) 459-64. Journal code: 0427043. ISSN: 0021-9673.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19980206
Entered Medline: 19911213

AB A method for the determination of histamine N-methyltransferase (HMT) activity by high-performance liquid chromatography based on post-column derivatization with omicron-phthalaldehyde is described. The determination involves the **separation** of the **substrate**, histamine, from its **product**. N tau-methylhistamine, using a weak **cation exchanger**, followed by on-line derivatization of these imidazoleamines with omicron-phthalaldehyde and their detection and quantitation with a fluorimetric detector. This assay method is suitable for the measurement of HMT activity during **enzyme** purification.

L19 ANSWER 35 OF 66 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 91:419759 SCISEARCH
THE GENUINE ARTICLE: FY044
TITLE: HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHIC DETERMINATION OF HISTAMINE N-METHYLTRANSFERASE ACTIVITY
AUTHOR: FUKUDA H; YAMATODANI A (Reprint); IMAMURA I; MAEYAMA K; WATANABE T; WADA H
CORPORATE SOURCE: OSAKA UNIV, FAC MED, DEPT PHARMACOL 2, NAKANOSHIMA 4-3-57, KITA KU, OSAKA 530, JAPAN; TOHOKU UNIV, SCH MED, DEPT PHARMACOL 1, AOBA KU, SENDAI, MIYAGI 980, JAPAN

Search completed by David Schreiber 308-4292

COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF CHROMATOGRAPHY-BIOMEDICAL APPLICATIONS, (1991) Vol. 567, No. 2, pp. 459-464.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 13

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A method for the determination of histamine N-methyltransferase (HMT) activity by high-performance liquid chromatography based on post-column derivatization with o-phthalaldehyde is described. The determination involves the **separation** of the **substrate**, histamine, from its **product**, N-tau-methylhistamine, using a weak **cation exchanger**, followed by on-line derivatization of these imidazoleamines with omicron-phthalaldehyde and their detection and quantitation with a fluorimetric detector. This assay method is suitable for the measurement of HMT activity during **enzyme** purification.

L19 ANSWER 36 OF 66 MEDLINE on STN DUPLICATE 20
ACCESSION NUMBER: 92059895 MEDLINE
DOCUMENT NUMBER: 92059895 PubMed ID: 1952051
TITLE: Determination of peptidylglycine alpha-amidating monooxygenase activity in human serum by thin-layer chromatography.
AUTHOR: Miyazaki N; Uemura T
CORPORATE SOURCE: Department of Radioisotope, Psychiatric Research Institute of Tokyo, Japan.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1991 Aug 15) 197 (1) 108-12. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19990129
Entered Medline: 19911206

AB We developed a simple assay system for the quantitative evaluation of peptidylglycine alpha-amidating monooxygenase activity using as substrate a 125I-labeled synthetic tripeptide, 125I-D-Tyr-Val-Gly, thin-layer chromatography, and a radiochromatoscanner. The basic principle of this method is that thin-layer chromatography separates the reaction product, 125I-D-Tyr-Val-NH2, from the substrate in an assay mixture. The 125I activities of both **substrate** and **product** **separated** from each other on a thin-layer chromatography plate were quantified with a radiochromatoscanner and the rate of conversion of the substrate to the product was calculated from their counts. Human serum was used as an **enzyme** source and the values of alpha-amidation activity obtained by our method under optimal conditions were almost identical to those of the published method using **ion** **-exchange** chromatography (sulphopropyl-Sephadex C-50 column) and a gamma-counter. Our method makes it possible to estimate the 10-pmol level of the product using 10 microliters of human serum and to assay a large number of samples rapidly and easily. It is therefore thought to be very useful for screening various tissues for alpha-amidation activity.

L19 ANSWER 37 OF 66 MEDLINE on STN DUPLICATE 21
ACCESSION NUMBER: 90253650 MEDLINE
DOCUMENT NUMBER: 90253650 PubMed ID: 2187485

Search completed by David Schreiber 308-4292

TITLE: Purification, assay and kinetic features of HIV-1 proteinase.
AUTHOR: Billich A; Hammerschmid F; Winkler G
CORPORATE SOURCE: Sandoz-Forschungsinstitut, Wien.
SOURCE: BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (1990 Mar) 371 (3) 265-72.
Journal code: 8503054. ISSN: 0177-3593.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199006
ENTRY DATE: Entered STN: 19900720
Last Updated on STN: 20000303
Entered Medline: 19900627

AB 1) The aspartic proteinase of the human immunodeficiency virus type 1 (HIV-1) was purified from cultures of recombinant E. coli. The **enzyme** preparation is homogeneous as judged by SDS-polyacrylamide gel electrophoresis and isoelectric focusing. 2) A rapid assay procedure for the proteinase was established which makes use of the cleavage of a radiolabeled decapeptide and the **separation** of **substrate** and labeled **product** by **ion-exchange** resin. 3) Activity of the **enzyme** is optimal at an ionic strength of 2.5-3.5M; also, the inhibitor pepstatin is a more potent inhibitor at higher ionic strength. This can be attributed to a tighter binding of both substrate and inhibitor in high-salt buffer. 4) The Km value of the decapeptide substrate is independent of the pH in the range of 3.5-7.5, while kcat shows a bell-shaped curve with a maximum at pH 5.2. The shape of the curve can be attributed to pKa values of 4.2 and 6.2 of groups on the **enzyme**. Pepstatin inhibition is optimal below pH 5.5, but becomes weak above pH 6.

L19 ANSWER 38 OF 66 MEDLINE on STN DUPLICATE 22
ACCESSION NUMBER: 90252969 MEDLINE
DOCUMENT NUMBER: 90252969 PubMed ID: 2339778
TITLE: **Assays** for amino acid decarboxylase **enzymes** using **ion-exchange** cartridges.
AUTHOR: Heerze L D; Kang Y J; Palcic M M
CORPORATE SOURCE: Department of Food Science, University of Alberta, Edmonton, Canada.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1990 Mar) 185 (2) 201-5.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199006
ENTRY DATE: Entered STN: 19900720
Last Updated on STN: 19980206
Entered Medline: 19900621

AB A general radiochemical method for estimating the activity of amino acid decarboxylases is reported. This method utilizes **ion-exchange** cartridges to **separate** unreacted radiolabeled amino acid **substrates** from **product** amines, which can then readily be quantitated by liquid scintillation counting. The assay is simple, rapid, and more sensitive than standard 14CO2 trapping procedures if uniformly labeled amino acid substrates are utilized. Acidic, basic, and aromatic amino acid decarboxylases can be assayed with

the appropriate choice of **cation** or **anion exchangers**. The utility of the method is demonstrated for aspartate-alpha-decarboxylase, tyrosine decarboxylase, and lysine decarboxylase where kinetic parameters are comparable to values obtained by standard radiochemical 14CO2 trapping assays.

L19 ANSWER 39 OF 66 MEDLINE on STN
ACCESSION NUMBER: 91023434 MEDLINE
DOCUMENT NUMBER: 91023434 PubMed ID: 2121061
TITLE: Improved assays of alpha-lactalbumin and galactosyltransferase.
AUTHOR: Holpert M; Cooper T G
CORPORATE SOURCE: Institute of Reproductive Medicine, University of Munster, Federal Republic of Germany.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1990 Jul) 188 (1) 168-75. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199011
ENTRY DATE: Entered STN: 19910117
Last Updated on STN: 19910117
Entered Medline: 19901107

AB The development and evaluation of a method for the determination of galactosyltransferase and alpha-lactalbumin activities using the addition of Dowex resin to the sample to **separate substrate** from **products** are described. For both assays galactosyltransferase activity was optimized by the addition of detergent, and relevant control incubations were included. The assay conditions were optimized for epididymal tissue and standards, and the assays were validated for accuracy and specificity with authentic bovine proteins and lactating rat mammary gland homogenates. Galactosyltransferase and alpha-lactalbumin activities in tissues were dependent on the extraction procedure used. Epididymal and testicular homogenates reduced the slopes of internal standards of galactosyltransferase but only testicular homogenates depressed slopes of internal standards of alpha-lactalbumin, necessitating the use of internal standards in the validation of the assays.

L19 ANSWER 40 OF 66 MEDLINE on STN DUPLICATE 23
ACCESSION NUMBER: 88207939 MEDLINE
DOCUMENT NUMBER: 88207939 PubMed ID: 3364735
TITLE: A radiochemical assay for a NADP+-specific gamma-glutamate semialdehyde dehydrogenase extracted from mitochondrial membrane of rat intestinal epithelial cells.
AUTHOR: Kramer J J; Gooding R C; Jones M E
CORPORATE SOURCE: University of North Carolina, Department of Biochemistry and Nutrition, Chapel Hill 27514.
CONTRACT NUMBER: AM35046 (NIADDK)
SOURCE: ANALYTICAL BIOCHEMISTRY, (1988 Feb 1) 168 (2) 380-6. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203

Search completed by David Schreiber 308-4292

Entered Medline: 19880531

AB A radiochemical assay has been developed for a NADP+-specific gamma-glutamate semialdehyde dehydrogenase from rat intestinal epithelial cells. The spectrophotometric **assay** utilized to **measure** the **enzyme** in bacterial cell homogenates is not sensitive enough for homogenates from rat mitochondria, which require an assay that can measure as little as 0.5 nmol NADPH formed/min/ml extract. The assay described here is sensitive to 0.1 nmol product formed/min/ml of extract and employs the use of [3H]pyrroline 5-carboxylate which is phosphorylated and oxidized by the **enzyme** to gamma-[3H]glutamyl phosphate, a product that decomposes to [3H]pyrrolidone 5-carboxylate. The latter **product** is **separated** from the **substrate** by **ion-exchange** chromatography. In order to correct for any product loss during separation by **ion-exchange** [14C]pyrrolidone 5-carboxylate is added as an internal standard to the deproteinized assay mixture. Under the assay conditions described mammalian gamma-glutamate semialdehyde dehydrogenase activity is linear with respect to time and protein concentration. Comparison between the kinetic parameters reported for the bacterial **enzyme** and those reported here for the mammalian **enzyme** indicate similarities in the pH optima as well as a requirement for phosphate. Kinetic studies on mammalian **enzyme** yield apparent Km values of 1.8 mM for pyrroline 5-carboxylate, 0.2 mM for NADP+, and 11.3 mM for phosphate.

L19 ANSWER 41 OF 66 EMBASE
on STN

ACCESSION NUMBER: 88142499 EMBASE
DOCUMENT NUMBER: 1988142499
TITLE: A method for determination of glucose 1,6-bisphosphatase.
AUTHOR: Bassols A.M.; Carreras J.; Cusso R.
CORPORATE SOURCE: Departament de Ciencies Fisiologiques Humanes i de la Nutricio, Unitat de Bioquimica, Facultat de Medecina, Universitat de Barcelona, Zona Universitaria de Pedralbes, 08028 Barcelona, Spain
SOURCE: Journal of Biochemical and Biophysical Methods, (1988) 16/1 (55-62).
ISSN: 0165-022X CODEN: JBBMDG
COUNTRY: Netherlands
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A sensitive and specific method to measure glucose 1,6-bisphosphatase activity, which allows the identification of the reaction products is described. [U-14C]Glucose 1,6-P2, synthesized by the glucose 1-P kinase activity of phosphofructokinase, is used as **substrate**. The reaction **products** are **separated** and identified by chromatography on **ion-exchange** paper.

L19 ANSWER 42 OF 66 MEDLINE on STN DUPLICATE 24

ACCESSION NUMBER: 88024936 MEDLINE
DOCUMENT NUMBER: 88024936 PubMed ID: 3117100
TITLE: Kinetic and structural characterization of reversibly inactivated beta-lactamase.
AUTHOR: Fink A L; Behner K M; Tan A K
CORPORATE SOURCE: Department of Chemistry, University of California, Santa Cruz 95064.
SOURCE: BIOCHEMISTRY, (1987 Jul 14) 26 (14) 4248-58.
Journal code: 0370623. ISSN: 0006-2960.

Search completed by David Schreiber 308-4292

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198712
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19871207

AB The reversible inhibition of beta-lactamase I from Bacillus cereus by cloxacillin, methicillin, and nafcillin has been systematically investigated. For these substrates the **enzymatic** reaction involves partitioning of the substrate between turnover and inhibition. Typically, concentrations of several hundred millimolar are necessary for complete inactivation. The completely inactivated **enzyme** could be formed by incubation at temperatures above 20 degrees C, where inhibition competes more effectively with turnover, and then stabilized by dropping the temperature to 0 degrees C or lower. The inactivated **enzyme** was rapidly **separated** from unreacted **substrate** and **product** at low temperature by centrifugal gel filtration or **ion exchange** and examined by far-UV circular dichroism for evidence of a conformational change. At pH 7 the inactivated **enzyme** had a secondary structure essentially identical with that of the native **enzyme**. The fluorescence emission spectrum of the inactivated **enzyme** (at pH 7) was also identical with that of the native **enzyme**. However, the inactivated **enzyme** was found to be considerably more sensitive to thermal denaturation, to acid-induced conformational isomerization, and to trypsinolysis than the native **enzyme**. We conclude from the circular dichroism results that the structure of the reversibly inactivated **enzyme** cannot be significantly different from that of the native **enzyme**. Therefore, previous findings that have been interpreted as indicating a major conformational change must be reevaluated. From examination of the low-resolution crystallographic structure of the **enzyme** we propose that the most likely cause of the inactivation is an alternate conformational state of the acyl-**enzyme** intermediate involving movement of one or more of the alpha-helices forming part of the active site. Such a structural effect could leave the secondary structure unchanged but have significant effects on the tertiary structure, catalysis, mobility, and susceptibility to trypsin and denaturation. We propose that the underlying physical reason why certain beta-lactam substrates bring about this "substrate-induced deactivation", or suicide inactivation, of the **enzyme** is due to the presence of the alternative acyl-**enzyme** conformation of similar free energy to the productive one, in which one (or more) essential catalytic group is no longer optimally oriented for catalyzing deacylation. Thus for substrates with a slow rate of deacylation (less than or equal to 100 s-1) the conformational transition can compete effectively on the time scale of the turnover reaction.

L19 ANSWER 43 OF 66 MEDLINE on STN DUPLICATE 25
ACCESSION NUMBER: 87211050 MEDLINE
DOCUMENT NUMBER: 87211050 PubMed ID: 2883912
TITLE: A specific radiochemical assay for pyrroline-5-carboxylate dehydrogenase.
AUTHOR: Small C; Jones M E
CONTRACT NUMBER: 5-RO1-AM35046-09 (NIADDK)
SOURCE: ANALYTICAL BIOCHEMISTRY, (1987 Mar) 161 (2) 380-6.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States

Search completed by David Schreiber 308-4292

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198706
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19970203
Entered Medline: 19870608

AB Previous studies of pyrroline-5-carboxylate dehydrogenase have been conducted using a spectrophotometric method to monitor substrate-dependent NAD(P)H production. For the **assay** of the mammalian **enzyme**, the spectrophotometric **assay** was found to be unacceptable for kinetic studies as the production of NAD(P)H was nonlinear with time and protein concentration. An assay which **measures** radiolabeled glutamate production by this **enzyme** in the presence of NAD+ from radiolabeled pyrroline-5-carboxylate has been developed. **Separation** of **substrate** from **product** is achieved by column chromatography using Dowex 50 **cation-exchange** resin. The product isolated by this procedure was identified as glutamate. This new assay is linear with time and protein concentration and gives reproducible results. The **assay** is not influenced by competing **enzyme** activities, such as glutamate dehydrogenase, in a liver homogenate so that quantitative conversion of pyrroline-5-carboxylate to glutamate is observed.

L19 ANSWER 44 OF 66 MEDLINE on STN DUPLICATE 26
ACCESSION NUMBER: 87134189 MEDLINE
DOCUMENT NUMBER: 87134189 PubMed ID: 3545892
TITLE: A practicable variant of the **ion exchange** method for the radiometric estimation of ornithine decarboxylase activity.
AUTHOR: Weber L W
SOURCE: EXPERIENTIA, (1987 Feb 15) 43 (2) 176-8.
Journal code: 0376547. ISSN: 0014-4754.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198704
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19970203
Entered Medline: 19870401

AB A known ornithine decarboxylase assay working with **ion exchange** separation of [3H]ornithine and [3H]putrescine has been revised. The assay can be performed in disposable 1.5 ml vessels with a total of four pipetting steps. The **separation** of **enzyme substrate** and **product**, respectively, requires 3 h per 50 samples. The detection limit is about 50 pmoles [3H]putrescine formed.

L19 ANSWER 45 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1984:294040 BIOSIS
DOCUMENT NUMBER: PREV198478030520; BA78:30520
TITLE: PROPYL AMINE TRANSFERASES IN CHINESE CABBAGE BRASSICA-PEKINENSIS CULTIVAR PAK-CHOY LEAVES.
AUTHOR(S): SINDHU R K [Reprint author]; COHEN S S
CORPORATE SOURCE: DEP PHARMACOLOGICAL SCIENCES, STATE UNIV NEW YORK STONY BROOK, STONY BROOK, NY 11794, USA
SOURCE: Plant Physiology (Rockville), (1984) Vol. 74, No. 3, pp. 645-649.

Search completed by David Schreiber 308-4292

CODEN: PLPHAY. ISSN: 0032-0889.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Spermidine synthase and spermine synthase activities in extracts of leaves of Chinese cabbage (B. pekinensis var. Pak Choy) were found and an assay of the former in crude extracts was developed. The method is based on the transfer of the propylamine moiety of decarboxylated S-adenosylmethionine to labeled putrescine, followed by **ion-exchange separation** of the labeled amine **substrate** and **product**, which are then converted to the 5-dimethylamino-1-napthalene sulfonyl (dansyl) derivatives and further purified and identified by thin layer chromatography. The specific radioactivity of putrescine present in the reaction mixture is determined, as is the radioactivity present in dansyl spermidine. The **enzyme** is also present in extracts of spinach leaves. Spermidine synthase was purified about 160-fold from Chinese cabbage leaves. After partial purification, a rapid coupled **enzymic assay** was used to study various properties of the **enzyme**. The plant **enzyme** shows maximum activity at pH 8.8 in glycine-NaOH buffer and has a MW of 81,000. The Km values for decarboxylated S-adenosylmethionine and putrescine are 6.7 and 32 μmol, respectively. The **enzyme** activity is inhibited strongly by dicyclohexylamine, cyclohexylamine, and S-adenosyl-3-thio-1, 8-diaminoctane. Dicyclohexylamine is the most potent inhibitor with an I50 at 0.24 μmol.

L19 ANSWER 46 OF 66 MEDLINE on STN DUPLICATE 27

ACCESSION NUMBER: 84295684 MEDLINE
DOCUMENT NUMBER: 84295684 PubMed ID: 6472496
TITLE: A coupled assay for histidine decarboxylase: in vivo turnover of this **enzyme** in mouse brain.
AUTHOR: Keeling D J; Smith I R; Tipton K F
SOURCE: NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY, (1984 Jun) 326 (3) 215-21.
Journal code: 0326264. ISSN: 0028-1298.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198410
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19841025

AB A sensitive coupled assay for histidine decarboxylase has been developed. This method involved conversion of [3H]histidine into [3H]histamine by the **enzyme** sample, with methylation of this product in situ, catalysed by the **enzyme** histamine N-methyltransferase, to yield [3H]N-tele-methylhistamine. The radioactive **product** was **separated** from the **substrate** by (i) extraction into chloroform, (ii) **ion-exchange** chromatography and (iii) liquid **cation-exchange** extraction. The "no tissue" assay blank comprised 0.0007% of the substrate radioactivity. Sample material with a histidine decarboxylase activity of as little as 0.14 fmol/min/ml (measured at 1 microM histidine) gave double the blank value. More than 50 assays could be performed in one day. This assay was used to determine the in vivo changes in mouse brain histidine decarboxylase activity following irreversible inhibition with (+) alpha-fluoromethylhistidine (alpha-FMH). From the time course of recovery of **enzyme** activity the half-life of histidine decarboxylase in vivo

was calculated to be 53 h.

L19 ANSWER 47 OF 66 MEDLINE on STN
ACCESSION NUMBER: 84174199 MEDLINE
DOCUMENT NUMBER: 84174199 PubMed ID: 6324484
TITLE: [Radiometric method of determining ATP: D-pantothenate-4'-phosphotransferase activity]. Radiometricheskii metod opredeleniia aktivnosti ATP: D-pantotenat-4'-fosfotransferazy.
AUTHOR: Khomich T I; Voskoboev A I; Chernikevich I P; Moiseenok A G
SOURCE: VOPROSY MEDITSINSKOI KHIMII, (1984 Jan-Feb) 30 (1) 131-2. Journal code: 0416601. ISSN: 0042-8809.
PUB. COUNTRY: USSR
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198405
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19840524

AB A radiometric procedure is developed for estimation of pantothenate kinase (EC 2.7.1.33) activity in various preparations of rat liver tissue; sodium 14C-D-pantothenate was used as a substrate and the reaction end product 4'-phosphopantothenic acid was measured. Optimal **separation** of the **substrate** and the end **product** was achieved by means of chromatography on DEAE-Sephadex A-25. 4'-phosphopantothenic acid was eluted from the column by 0.4 N HCl thus avoiding the label dilution and possible quenching of scintillation.

L19 ANSWER 48 OF 66 MEDLINE on STN DUPLICATE 28
ACCESSION NUMBER: 84116737 MEDLINE
DOCUMENT NUMBER: 84116737 PubMed ID: 6420632
TITLE: A sensitive radiometric assay for enkephalin convertase and other carboxypeptidase B-like enzymes.
AUTHOR: Stack G; Fricker L D; Snyder S H
CONTRACT NUMBER: DA-00074 (NIDA)
DA-00266 (NIDA)
NS-16375 (NINDS)
+
SOURCE: LIFE SCIENCES, (1984 Jan 9) 34 (2) 113-21. Journal code: 0375521. ISSN: 0024-3205.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198403
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 20000303
Entered Medline: 19840312

AB A sensitive radiometric **assay** for carboxypeptidase B-like **enzymes** has been developed using enkephalin convertase, an enkephalin synthesizing carboxypeptidase. The assay is based on the differential solubility of 3H-labeled substrate and product in chloroform. The substrates 3H-benzoyl-Phe-Ala-Arg or 3H-benzoyl-Phe-Leu-Arg are poorly soluble in chloroform due to the **charged** arginine. The products of carboxypeptidase B-like activity on these substrates, 3H-benzoyl-Phe-Ala or 3H-benzoyl Phe-Leu partition quantitatively into chloroform, allowing rapid **separation** of **product** from **substrate**. This assay is approximately 100 times more sensitive

than a similar fluorometric assay utilizing dansyl-Phe-Ala-Arg as a substrate.

L19 ANSWER 49 OF 66 MEDLINE on STN DUPLICATE 29
ACCESSION NUMBER: 82205994 MEDLINE
DOCUMENT NUMBER: 82205994 PubMed ID: 6805462
TITLE: Spermidine oxidase in human pregnancy serum. Probable identity with diamine oxidase.
AUTHOR: Gahl W A; Vale A M; Pitot H C
CONTRACT NUMBER: CA 22484 (NCI)
SOURCE: BIOCHEMICAL JOURNAL, (1982 Jan 1) 201 (1) 161-6.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198207
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 20021210
Entered Medline: 19820708

AB Diamine oxidase was previously measured in human pregnancy serum with putrescine or histamine as substrate. We have now documented the presence of spermidine oxidase activity in pregnancy serum by means of a specific radioactive assay with [14C]spermidine as substrate and Dowex 50 **cation-exchange** chromatography to **separate products** from **substrate**. The apparent Km of a partially purified preparation of this **enzyme** for spermidine was 10.9 microM and the Ki for aminoguanidine was 0.8 microM. The pH optimum (pH 9.0) and temperature optimum (55 degrees C) were identical with those for diamine oxidase. Spermidine oxidase activity and diamine oxidase activity eluted in a concerted fashion when pregnancy serum was subjected to cadaverine-Sepharose chromatography, gel filtration and **ion-exchange** chromatography. Spermidine oxidase became detectable in serum during pregnancy in the human approx. 8 weeks after the last menstrual period and increased with gestational age in concert with the increase in diamine oxidase activity, reaching a plateau at 20 weeks of gestation. Foetal-cord serum displayed virtually no activity of either **enzyme**. A 400-fold-purified preparation of diamine oxidase retained the same diamine oxidase/spermidine oxidase ratio as exhibited by crude pregnancy serum. These data suggest that in pregnancy serum, unlike foetal bovine serum, spermidine oxidase and diamine oxidase activity may be a single **enzyme** protein.

L19 ANSWER 50 OF 66 MEDLINE on STN DUPLICATE 30
ACCESSION NUMBER: 82242184 MEDLINE
DOCUMENT NUMBER: 82242184 PubMed ID: 7047758
TITLE: Choline acetyltransferase: further studies on the reverse reaction.
AUTHOR: Hsu L L; Chao L P
CONTRACT NUMBER: NS-11087 (NINDS)
SOURCE: JOURNAL OF NEUROSCIENCE RESEARCH, (1982) 7 (2) 155-62.
Journal code: 7600111. ISSN: 0360-4012.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198209
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19980206

Search completed by David Schreiber 308-4292

Entered Medline: 19820910

AB In order to further characterize the reaction mechanism of brain ChAc in its purified form, we have investigated the reverse reaction of ChAc in terms of pH optimum, salt effects, and substrate kinetics using a radiochemical assay. We directly measured the reaction **product** acetylcoenzyme A which was **separated** from the **substrate** ACh by a **cation exchange** column. Dowex 50W-X8 (Na+ form). The reverse reaction of ChAc was linear with incubation time up to 40 minutes, and with **enzyme** protein concentration up to 5 micrograms. It had a pH optimum at 7.0. At 0.22 M the monovalent chloride and bromide salts activated the reverse ChAc activity by 23-47% but the fluoride and iodide salts inhibited the reverse **enzyme** activity by 10-30%. Kinetic studies in the absence of salt showed that KACh was 0.62 +/- 0.06 mM, KCoA . SH was 12.68 +/- 1.21 microM, and Vmax was 11.6 +/- 1.0 nmol AcCoA/mg protein/min. These data are in disagreement with the values reported on partially purified ChAc from bovine brain by Glover and Potter [1971] and Hersh [1980]. This indicates that further investigations are necessary to clarify or resolve these differences.

L19 ANSWER 51 OF 66 MEDLINE on STN DUPLICATE 31
ACCESSION NUMBER: 82026037 MEDLINE
DOCUMENT NUMBER: 82026037 PubMed ID: 6269782
TITLE: A highly sensitive fluorimetric assay for pyridoxal phosphate phosphatase.
AUTHOR: Smith G P; Smith G D; Peters T J
SOURCE: CLINICA CHIMICA ACTA, (1981 Aug 10) 114 (2-3) 257-62. Journal code: 1302422. ISSN: 0009-8981.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198112
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19900316
Entered Medline: 19811215

AB A highly sensitive fluorimetric assay for pyridoxal phosphate phosphatase is described. The assay involves **separation** of the **substrate** and **product** by **ion-exchange** chromatography followed by treatment of pyridoxal with potassium cyanide under slightly alkaline conditions to form 4-pyridoxolactone, a highly fluorescent compound. Certain kinetic properties of the **enzyme** activities in human neutrophils are described.

L19 ANSWER 52 OF 66 MEDLINE on STN DUPLICATE 32
ACCESSION NUMBER: 81239945 MEDLINE
DOCUMENT NUMBER: 81239945 PubMed ID: 6166629
TITLE: Action pattern of human pancreatic alpha-amylase on maltoheptaose, a substrate for determining alpha-amylase in serum.
AUTHOR: Haegele E O; Schaich E; Rauscher E; Lehmann P; Grassl M
SOURCE: JOURNAL OF CHROMATOGRAPHY, (1981 Apr 10) 223 (1) 69-84. Journal code: 0427043. ISSN: 0021-9673.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198109
ENTRY DATE: Entered STN: 19900316

Search completed by David Schreiber 308-4292

Last Updated on STN: 19970203
Entered Medline: 19810915

AB An **enzymatic assay** for the determination of alpha-amylase in serum was developed which employed a soluble substrate, maltoheptaose, and a coupled **enzymatic** indicator reaction consisting of alpha-glucosidase and the hexokinase-glucose-6-phosphate dehydrogenase system. We used high-performance liquid chromatography (HPLC) to establish the action pattern of maltoheptaose under the test conditions: (A) the action pattern of alpha-amylase, (B) that of the combined action of alpha-amylase and alpha-glucosidase. Conductive to this effect was: the availability of pure maltoheptaose and human pancreatic alpha-amylase; the development of an adequate procedure for sample pretreatment (partition chromatography on a mixed-bed **ion exchange**) and of an HPLC system for **separation** of **substrate** and reaction **products** without interference from by products of the assay (partition chromatography on a **cation-exchange** column with acetonitrile-water); and the use of a new, very sensitive refractometric detector revealing sugar amounts as low as 40 ng. We derived the following stoichiometric equations: (see formula index).

L19 ANSWER 53 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 33
ACCESSION NUMBER: 1981:235556 BIOSIS
DOCUMENT NUMBER: PREV198172020540; BA72:20540
TITLE: RADIOCHEMICAL ESTIMATION OF SERUM POLY AMINE OXIDASE ACTIVITY IN HUMAN PREGNANCY.
AUTHOR(S): MORGAN D M L [Reprint author]; ILLEI G
CORPORATE SOURCE: DIV OF PERINATAL MED, CLINICAL RES CENT, WATFORD RD, HARROW HA1 3UJ, MIDDLESEX, UK
SOURCE: Medical Laboratory Sciences, (1981) Vol. 38, No. 1, pp. 49-56.
CODEN: MLASDU. ISSN: 0308-3616.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB A method is presented for estimation of polyamine oxidase activity in human serum in which the sample is incubated with 14C-labeled polyamine and, after deproteinization with trichloracetic acid, **products** are **separated** from unreacted **substrate** by chromatography on a strong **cation exchange** resin. The radioactivity in each fraction is then determined by liquid scintillation counting. Product formation is linear with amount of sample (10-100 µl) and incubation time (up to 90 min). Triplicate assays of 30 sera gave a within-batch coefficient of variation of 7% (range 196-344 nmol/min per l, SD 19.7). Serum polyamine oxidase activity was found to increase significantly with time and showed an approximately linear trend from the 10th wk of pregnancy onwards, although there was considerable individual variation. No activity was found in male sera and only very low activity in sera from non-pregnant females of reproductive age.

L19 ANSWER 54 OF 66 MEDLINE on STN DUPLICATE 34
ACCESSION NUMBER: 80265174 MEDLINE
DOCUMENT NUMBER: 80265174 PubMed ID: 7406861
TITLE: Separation of putrescine oxidase and spermidine oxidase in foetal bovine serum with the aid of a specific radioactive assay of spermidine oxidase.
AUTHOR: Gahl W A; Vale A M; Pitot H C
SOURCE: BIOCHEMICAL JOURNAL, (1980 Apr 1) 187 (1) 197-204.

Search completed by David Schreiber 308-4292

Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198010
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 20021210
Entered Medline: 19801021

AB 1. A sensitive and specific assay for spermidine oxidase is described. The method involves the **separation** of [14C]spermidine (**substrate**) from [14C]putrescine (**product**) and other 14C-labelled products on a Dowex 50 **cation-exchange** column: 92% of the putrescine applied to the column was eluted by 2.3 M-HCl, but this treatment left 96% of the spermidine bound to the column. Unchanged spermidine could be removed from the column by elution with 6 M-HCl. 2. By means of this assay, foetal and adult bovine serum were each shown to contain spermidine oxidase activity, putrescine being a major product of the oxidation of spermidine by the serum **enzymes**. 3. In foetal bovine serum, spermidine oxidase activity is separable from putrescine oxidase activity by chromatography on a cadaverine-Sephadex column, by gel filtration and by **ion-exchange** column chromatography. Putrescine oxidase was purified 1900-fold and spermidine oxidase 130-fold by these procedures. The former oxidized putrescine but not spermidine, and spermidine oxidase exhibited no activity with putrescine as substrate.

L19 ANSWER 55 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1979:270721 BIOSIS
DOCUMENT NUMBER: PREV197968073225; BA68:73225
TITLE: DIALYSIS CONTINUOUS PROCESS FOR AMMONIUM LACTATE FERMENTATION IMPROVED MATHEMATICAL MODEL AND USE OF DE PROTEINIZED WHEY.
AUTHOR(S): STIEBER R W [Reprint author]; GERHARDT P
CORPORATE SOURCE: DEP MICROBIOL PUBLIC HEALTH, MICH STATE UNIV, EAST LANSING, MICH 48824, USA
SOURCE: Applied and Environmental Microbiology, (1979) Vol. 37, No. 3, pp. 487-495.
CODEN: AEMIDF. ISSN: 0099-2240.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB **Separate** terms for **substrate** limitation and **product** inhibition were incorporated into an equation describing the rate of cell growth for the steady-state fermentation of lactose to lactic acid with neutralization to a constant pH by NH3. The equation was incorporated into a generalized mathematical model of a dialysis continuous process for the fermentation, developed previously, in which the substrate is fed into the fermentor and the fermentor contents are dialyzed through a membrane against water. The improved model was used to simulate the fermentation on a digital computer, and the results agreed with previous experimental tests using whole whey as the substrate. Further simulations were then made to guide experimental tests using deproteinized whey as the substrate. Dried cheese-whey ultrafiltrate was rehydrated with tap water to contain 242 mg of lactose/ml, supplemented with 8 mg of yeast extract/ml, **charged** into a 5 l fermentor without sterilization, adjusted in pH (5.5) and temperature (44° C), and inoculated with an adapted culture of Lactobacillus bulgaricus. The fermentor and dialysate circuits were connected, and a series of

steady-state conditions was managed nonaseptically for 71 days. The fermentation of deproteinized whey relative to whole whey, with both highly concentrated, resulted in similar extents of product accumulation but at a lesser rate.

L19 ANSWER 56 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 35
ACCESSION NUMBER: 1979:268905 BIOSIS
DOCUMENT NUMBER: PREV197968071409; BA68:71409
TITLE: A RAPID AND SENSITIVE ASSAY FOR NEURAMINIDASE EC-3.2.1.18 APPLICATION TO CULTURED FIBROBLASTS.
AUTHOR(S): FRISCH A [Reprint author]; NEUFELD E F
CORPORATE SOURCE: NATL INST HEALTH, ROOM 9N-238, BUILD 10, 9000 ROCKVILLE PIKE, BETHESDA, MD 20014, USA
SOURCE: Analytical Biochemistry, (1979) Vol. 95, No. 1, pp. 222-227.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB Neuraminidase [EC 3.2.1.18] substrates of high specific activity (> 300 μCi/μmol) were prepared by reduction of sialyllactose with tritiated NaBH4, followed by separation of the 2 → 3 and 2 → 6 isomers of [3H]sialyllactitol by paper chromatography. Hydrolysis of sialyllactitol by neuraminidase was monitored by measuring the radioactivity in the neutral reaction **product**, which was **separated** from the **charged substrate** by passage over a small **anion exchange** column. The assay was applied to the neuraminidase activity of cultured human skin fibroblasts. The Km was 1.1 mM for both substrates, the pH optimum was 4.0 and 2 → 3 isomer was hydrolyzed twice as fast as the 2 → 6. In several genetic disorders associated with neuraminidase deficiency, the activity toward both isomers was reduced almost completely (mucolipidoses I and II; Goldberg syndrome), or only partially (mucolipidosis III; adult myoclonus syndrome); however, the relative activity towards the 2 isomers remained approximately the same in all cases.

L19 ANSWER 57 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1979:128624 BIOSIS
DOCUMENT NUMBER: PREV197967008624; BA67:8624
TITLE: SOME RADIOMETRIC MICRO ASSAYS FOR BUTYRYL CHOLIN ESTERASE EC-3.1.1.8.
AUTHOR(S): MADERDRUT J L [Reprint author]
CORPORATE SOURCE: NEUROBIOL CURRIC, UNIV NC SCH MED, CHAPEL HILL, NC 27514, USA
SOURCE: Analytical Biochemistry, (1978) Vol. 88, No. 2, pp. 406-416.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB A simple method for purifying [14C]butyrylcholine and incubation conditions suitable for estimating butyrylcholinesterase [EC 3.1.1.8] activity are described. Solvent extraction and **anion-** and **cation-exchange** procedures for separating butyrylcholine from butyric acid are described and compared. The **anion-exchange** method provides the most efficient **separation** of **precursor** from **product**. Blanks using the

anion-exchange method average approximately 0.1% of the total radioactivity in the substrate; product recovery is quantitative. Butyrylcholinesterase activity can be measured reliably in samples of embryonic nervous system tissue having wet weights less than 5 μg.

L19 ANSWER 58 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1979:126810 BIOSIS
DOCUMENT NUMBER: PREV197967006810; BA67:6810
TITLE: LABELING OF SMALL MOLECULES WITH FLUORESCEIN.
AUTHOR(S): SIMPSON I [Reprint author]
CORPORATE SOURCE: DEP PHYSIOL BIOPHYS, UNIV MIAMI SCH MED, MIAMI, FLA 33152, USA
SOURCE: Analytical Biochemistry, (1978) Vol. 89, No. 1, pp. 304-305.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB The finding that fluorescein passes through a cell junction of low electrical resistance, has led to the synthesis of other fluorescent molecules for probing junctional permeability. These new probes combined some of the desirable features of fluorescein, namely a high fluorescence yield, excitation by visible light, low toxicity toward cells and lack of nonjunctional membrane transport, with some nonfluorescent parameters, e.g., size and **charge**. Thus fluorescein isothiocyanate (FITC) which has been widely used in immunochemistry to label proteins was coupled to some smaller substances of well-defined structure such as amino acids. Although the actual coupling of FITC to small **substrates** presents no problem, the **separation** of the coupled **product** from the hydrolysed dye can be a major difficulty. Details of a simple chromatographic method for carrying out this separation are described, and the method is illustrated with insulin A chain.

L19 ANSWER 59 OF 66 MEDLINE on STN
ACCESSION NUMBER: 78237492 MEDLINE
DOCUMENT NUMBER: 78237492 PubMed ID: 98244
TITLE: A sensitive procedure for the diagnosis of N-acetyl-galactosamine-6-sulfate sulfatase deficiency in classical Morquio's disease.
AUTHOR: Glossl J; Kresse H
SOURCE: CLINICA CHIMICA ACTA, (1978 Aug 15) 88 (1) 111-9.
Journal code: 1302422. ISSN: 0009-8981.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197810
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19980206
Entered Medline: 19781025
AB The trisaccharide 6-sulfo-N-acetylgalactosamine-glucuronic acid-6-sulfo-N-acetyl-[1-3H]galactosaminitol was used as a substrate for the determination of N-acetylgalactosamine-6-sulfate sulfatase activity. The amount of liberated sulfate was measured indirectly by **separating** monosulfated reaction **products** from the **substrate** on Dowex 1 X 2 microcolumns in a simple two step procedure. Fibroblast homogenates from patients with various genotypes, except classical Morquio's disease, released 410 +/- 90 pmol sulfate/h/mg

cell protein. The **enzyme** exhibited a pH optimum of pH 4.8 and a KM of about 1 X 10(-4) mol/1. It was strongly inhibited by phosphate, sulfate and chloride ions. In three cell lines from patients with classical Morquio's disease a residual activity between 1 and 2% of the mean normal activity was found. All cell lines tested released sulfate from 6-sulfo-N-acetylglucosamine-glucuronic acid-[1-3H]-anhydromannitol. Cell extracts from cultured amniotic fluid cells exhibited a N-acetylgalactosamine-6-sulfate sulfatase activity between 120 and 320 pmol/h/mg protein. An **enzyme** activity of 370 +/- 100 pmol sulfate/h/mg protein was found in peripheral leucocytes from healthy donors. The determination of N-acetyl-galactosamine-6-sulfate sulfatase activity in one family with an affected patient indicated that the **enzyme** deficiency is also expressed in leucocytes.

L19 ANSWER 60 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 36
ACCESSION NUMBER: 1978:212722 BIOSIS
DOCUMENT NUMBER: PREV197866025219; BA66:25219
TITLE: **ENZYMATIC** SYNTHESIS OF 6 CARBON-14 OROTIDINE 5 MONO PHOSPHATE AND ITS USE IN THE ASSAY OF OROTATE PHOSPHO RIBOSYL TRANSFERASE EC-2.4.2.10 AND OROTIDYLATE DECARBOXYLASE EC-4.1.1.23.
AUTHOR(S): RAWLS J M JR [Reprint author]
CORPORATE SOURCE: TH MORGAN SCH BIOL SCI, UNIV KY, LEXINGTON, KY 40506, USA
SOURCE: Analytical Biochemistry, (1978) Vol. 86, No. 1, pp. 107-117.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB A rapid and efficient method is described for the synthesis of [6-14C]orotidine 5'-monophosphate from radioactive orotic acid using purified yeast orotate phosphoribosyltransferase and inorganic pyrophosphatase. Radioactive orotidine 5'-monophosphate is purified by **ion exchange** chromatography and employed in small scale assays of Drosophila orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidylate decarboxylase (EC 4.1.1.23) in which both **enzyme** activities are simultaneously **measured** in single reaction mixtures. Radioactive **substrate** and **products** are **separated** for counting using DEAE-cellulose paper chromatograms developed in 1 or 2 solvents.

L19 ANSWER 61 OF 66 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1978:226640 BIOSIS
DOCUMENT NUMBER: PREV197866039137; BA66:39137
TITLE: A RADIOMETRIC **ANION EXCHANGE** METHOD FOR ACETYL CHOLIN ESTERASE EC-3.1.1.7.
AUTHOR(S): MADERDRUT J L [Reprint author]
CORPORATE SOURCE: NEUROBIOL CURRIC, UNIV NC SCH MED, CHAPEL HILL, NC 27514, USA
SOURCE: Neurochemical Research, (1977) Vol. 2, No. 6, pp. 717-722.
CODEN: NEREDZ. ISSN: 0364-3190.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB An **anion-exchange** method for estimating chick spinal chord and mouse brain AChE [acetylcholinesterase, EC 3.1.1.7] activity is described which provided both quantitative product recovery and higher sensitivity than the solvent extraction method. Direct comparison with

the solvent extraction procedure on 3 seperate occassions has yielded a higher experimental to blank ratio (higher sensitivity) for the **anion-exchange** procedure. The **anion-exchange** method is approximately 6 times more efficient than the **cation-exchange** method, and 2-3 times more efficient than the solvent extraction method in **separating precursor** from **product**. The **anion-exchange** method has the dual advantages of substantially higher sentivitity and greater specificity.

L19 ANSWER 62 OF 66 MEDLINE on STN DUPLICATE 37
ACCESSION NUMBER: 76232306 MEDLINE
DOCUMENT NUMBER: 76232306 PubMed ID: 181064
TITLE: Some characteristics of sn-glycero-3-phosphocholine diesterases from rat brain.
AUTHOR: Abra R M; Quinn P J
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1976 Jun 22) 431 (3) 631-9. Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197610
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19970203
Entered Medline: 19761002

AB 1. sn-Glycero-3-phosphocholine diesterase activities, glycerophosphohydrolase (EC 3.1.4.2) and choline phosphohydrolase (EC 3.1.4.38) from rat brain have been partially purified and characterized using sn-glycere-3-[32P]phosphocholine as **substrate** and **separating** the reaction **products** by **anion-exchange** chromatography and **ionophoresis**. 2. Rat brain contained particulate (75%) and soluble (25%) activity from both diesterases. No difference in pH optimum or metal ion requirement for the particulate compared to the soluble **enzymes** was observed. 3. Glycerophosphohydrolase (EC 3.1.4.2) was purified 60-fold, choline phosphohydrolase (EC E.1.4.38) 120-fold from rat brain supernatant fraction by DEAE-cellulose **ion-exchange** chromatography and sucrose density gradient centrifugation. The density gradient results in conjunction with dodecyl sulphate-polyacrylamide gel disc electrophoresis yielded molecular weight estimates of 230 000 (monomer 62 000) for choline phosphohydrolase and 120 000 (monomer 70 000) for glycerophosphohydrolase (EC 3.1.4.2). 4. Glycerophosphohydrolase (EC 3.1.4.2) has a pH optimum of 8.9 and a Km for sn-glycero-3-phosphocholine of 0.6 mM. The **enzyme** is inhibited by EDTA and reactivated by Ca2+. Choline phosphohydrolase (EC 3.1.4.38) has pH optimum 10.5, a Km of 2 mM and is unaffected by EDTA. Both **enzymes** require Ca2+ for maximum activity.

L19 ANSWER 63 OF 66 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 77048440 EMBASE
DOCUMENT NUMBER: 1977048440
TITLE: A rapid radiometric assay for adenosine deaminase.
AUTHOR: Jackson R.J.; Keightley R.G.
CORPORATE SOURCE: Dept. Microbiol., Univ. Alabama, Birmingham, Ala. 35294, United States
SOURCE: Analytical Biochemistry, (1976) 70/2 (403-412). CODEN: ANBCA2

Search completed by David Schreiber 308-4292

DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
023 Nuclear Medicine
LANGUAGE: English
AB A rapid radiochemical procedure for the measurement of adenosine deaminase is described. The method employs phospho Sephadex, a weak **cation exchanger**, which permits the **enzymic** product inosine to pass unretarded through the gel while the radioactive substrate adenosine is retained. Use of a Millipore filter manifold permits rapid processing of samples and eliminates time consuming column chromatographic, electrophoretic, or paper chromatographic techniques required for **separation** of **product** and **substrate**. The activity of adenosine deaminase was examined in spleen cell preparations prepared from normal CBA mice. Excellent agreement of results was obtained when the radioactive method was compared with two other independent assay techniques.

L19 ANSWER 64 OF 66 MEDLINE on STN
ACCESSION NUMBER: 76067485 MEDLINE
DOCUMENT NUMBER: 76067485 PubMed ID: 297
TITLE: Ecdysone Oxidase, an **enzyme** from the blowfly Calliphora erythrocephala (Meigen).
AUTHOR: Koolman J; Karlson P
SOURCE: HOPPE-SEYLERS ZEITSCHRIFT FUR PHYSIOLOGISCHE CHEMIE, (1975 Jul) 356 (7) 1131-8.
Journal code: 2985060R. ISSN: 0018-4888.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197602
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19970203
Entered Medline: 19760209
AB In the blowfly, the formation of 3-dehydroecdysone from the insect molting hormone ecdysone is catalyzed by an **enzyme** which carries hydrogen from ecdysone and ecdysterone to oxygen. The **enzyme** is therefore called "ecdysone oxidase". Two methods are described for the detection of ecdysone oxidase activity, one using a radiolabelled **substrate** which is **separated** from the **product** by thin-layer chromatography after the reaction, and the other using dichloroindophenol, which is discoloured by the redox reaction. The ecdysone oxidase is purified by a factor of 2200 from prepupae of Calliphora erythrocephala using salt precipitation and **ion exchange** chromatography. The ecdysone oxidase has a Km value for ecdysone of 42muM. The pH optimum is 6.5. The temperature optimum lies at 45 degrees C. The ecdysone oxidase has a molecular weight of 240000.

L19 ANSWER 65 OF 66 EMBASE on STN
ACCESSION NUMBER: 74025412 EMBASE
DOCUMENT NUMBER: 1974025412
TITLE: Assay for S adenosylmethionine: methionine methyltransferase.
AUTHOR: Allamong B.D.; Abrahamson L.
CORPORATE SOURCE: Dept. Biol., West Virginia Univ., Morgantown, W.Va. 26506, United States
SOURCE: Analytical Biochemistry, (1973) 53/2 (343-349).
CODEN: ANBCA2

Search completed by David Schreiber 308-4292

Gitomer 09/888,008

DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
023 Nuclear Medicine
LANGUAGE: English
AB A quantitative assay for S adenosylmethionine: methionine methyltransferase in phosphate buffer extracts has been developed. This **enzyme** catalyzes the biosynthesis of S methylmethionine from methionine and S adenosylmethionine. The radioactively labeled **product**, S methylmethionine, is first **separated** from the radioactively labeled **substrate**, L methionine, by means of **ion exchange** chromatography. Once separated thusly, the amount present can then be directly determined by the use of a liquid scintillation spectrometer.

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ACCESSION NUMBER: 74074834 EMBASE
DOCUMENT NUMBER: 1974074834
TITLE: Assay of nanogram levels of triglyceride lipase with a radioactive substrate.
AUTHOR: Marsh W.H.; Fitzgerald P.J.
CORPORATE SOURCE: Dept. Pathol., State Univ. New York, Downstate Med. Cent., Brooklyn, N.Y. 11203, United States
SOURCE: Journal of Lipid Research, (1972) 13/2 (284-287).
CODEN: JLPRAW
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
023 Nuclear Medicine
LANGUAGE: English
AB A simple, sensitive procedure for the determination of triglyceride lipase activity has been developed. Nanogram amounts of oleic acid hydrolyzed from commercially available [14C]triolein were readily determined by the counting of the radioactivity of substrate and product after their rapid chromatographic separation on copper hydroxide impregnated **ion exchange** paper. Comparison of the relative amounts of radioactivity of the **separated substrate** and **product** gave an estimate of the percentage of hydrolysis of substrate. Comparison of results with a standard of pure lipase enables one to express the amount of hydrolysis in terms of the standard lipase. The results show that measured activity is a linear function of time up to 1 hr of incubation and of amounts of **enzyme** up to 125 ng. Reproducibility of the test is good.

Search completed by David Schreiber 308-4292